VESSEL WALL REACTIONS TO ENDOVASCULAR STENT IMPLANTATION

VAATWAND REAKTIES
OP ENDOVASCULAIRE STENT IMPLANTATIE

PROEFSCHRIFT

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Cover illustration: A schematic representation of the early events after stenting.

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CONTENTS

Chapter 1
General Introduction

Stents, What are they.

Stents are tubular prostheses that are meant to provide an inner mechanical support for hollow structures such as the oesophagus, trachea, bile ducts, ureters, veins and arteries. They can even be used to create venous shunts (intrahepatic portacaval stent shunts [1]) or to close arteriovenous fistulae. In infants with some forms of congenital heart malformation, stents can be used to maintain a patent ductus arteriosus.

Stents, usually placed via transcatheter implantation, are available as self-expanding structures and balloon-expandable structures (see also Table I). Self expanding stents can act as a spring or make use of temperature changes in case of shape-memory metals. Balloon-expandable stents depend on inflatable balloons for creating their final size.
In this thesis the role of stents as endovascular support devices is discussed.

History of Stents in a Nutshell.

The idea of introducing endovascular support devices into the lumen of a vessel is not new. As early as 1912 the Nobel prize winner A. Carrel[2] reports the results of the permanent intubation of the thoracic aorta of dogs with aluminium tubes. He was inspired by the work of his colleague Abbe who united the cut ends of the aorta of a cat with glass tubing. Four months after surgery, Abbe presented the cat in good condition at the Academy of Medicine. The cat escaped afterwards.

It was not until 1969 however, that C.T. Dotter[3] gave new input to this idea. In an attempt to improve transluminal recanalization of completely occluded arteries, Dotter developed tubular prostheses but soon found that all impervious grafts occluded within 24 hours. This led to the idea of a coil spring equivalent. The results were inspiring.

The first human coronary stent implantations were performed in 1986[4] by J. Puel (Toulouse, France) and U. Sigwart (Lausanne, Switzerland). The Thoraxcenter Rotterdam followed in november of that year. Since then, many designs and materials have been used for this type of device, resulting in a variety of stents available for experimental and clinical evaluation[5].

Rationale for Stenting and Current Clinical Use.

Stents for endovascular applications are used in adjunct to or as a replacement for conventional percutaneous transluminal coronary angioplasty (PTCA). Indications proposed for stenting are for instance the improvement of angiographic results after PTCA or elective stenting to increase long-term patency (reduce restenosis). Stents can also be used as a rescue or bail-out device in case of acute or threatened closure such as dissection following PTCA or diagnostic procedures. Although stents are widely available commercially for peripheral applications the use of most coronary stents is still restricted to certain indications, this situation, however, might change rapidly in the near future. Multicenter trials with preset criteria for patient eligibility and management are presently performed to assess their efficacy. BENESTENT[6] for instance is an international multicenter trial comparing elective stenting to balloon angioplasty, with STRESS as its American counterpart. GRACE international (Gianturco-Roubin stent Acute Closure Evaluation) is aimed at comparing stenting with prolonged and repeated balloon angioplasty for the management of acute or threatened closure resulting from interventional procedures.

Table I Design and characteristics of stents in clinical evaluation. Modified from De Jaegere et al [8].

Stent	Configuration	Filament composition	Filament thickness (mm)	Stent diameter (mm)	Stent lenght (mm)	surface area (%)
Self-expanding						
Wallstent	braided mesh	stainless steel	0.07-0.1	3.5-6.0	21-45	18.5-20
Balloon-expandable						
Palmaz-Schatz	slotted tube	stainless steel	0.08	3.0-4.0	15	10
Gianturco-Roubin	incomplete coil	stainless steel	0.15	2.0-4.0	20	10
Wiktor	helical coil	tantalum	0.125	3.0-4.5	15-17	5-10
Strecker	knitted wire	stainless steel/ tantalum	0.07	2.0-3.5	15-25	___

Prerequisites for Endovascular Stents.

Theoretically, an ideal stent should comply with the following criteria[9]:

1. It should have sufficient radial force to maintain an appropriate lumen, but without inducing barotrauma.

2. It should remain fixed at the preselected site without migration.
3. It should be non-thrombogenic.
4. It should not induce a chronic inflammatory or foreign body response.
5. It should not delay wound healing (e.g. endothelialization) nor induce excessive hyperplasia.

Most of these criteria speak for themselves, as the intended goal of the treatment is to create an appropriate lumen. Any adverse effect such as barotrauma (1, too much force) which can cause medial necrosis; migration of the device (2, not enough force); thrombogenicity (3); chronic inflammation (can delay wound healing and induce thrombus formation, 4,5); and excessive intimal hyperplasia (5) should be avoided as all can ultimately result in re-narrowing of the treated lesion.

Problems with Metal Stents.

The first problem that is encountered is thrombogenicity. In patients (sub)acute thrombotic occlusion occurs in 2% - 10% of all cases [10,11,12] and necessitates a stringent anticoagulant regimen consisting of antiplatelet therapy (e.g. acetylsalicylic acid and dipyridamol) and anticoagulant therapy (e.g. heparin, coumadin) and often also dextran. Consequently bleeding complications can occur in up to 20 - 30% of cases [10,11]. As suggested in Chapter 6, thrombotic events take place in every case, successful or not. Persistence of thrombus remnants could have a prolonged influence as a source for growth factors, chemotactic factors etc. It is therefore important to improve blood compatibility for stents.

Another problem is that although stents may reduce the incidence of restenosis, they do no prevent it [11], which indicates that tissue compatibility should perhaps be improved.

Can we Change Stent Behaviour.

The behaviour of a material as a vascular implant is dictated by the acute and chronic response to blood as well as the response of the vascular tissue to the foreign material[13]. The thrombotic response for instance is dictated by surface characteristics such as charge [14], chemistry [15] and topography (i.e. roughness, porosity, gas microbubbles) which determine not only pro and anticoagulant activity, but also complement [16] and leucocyte activation [17].

The local tissue response is again dictated by surface charge, chemistry and topography, but additionally by the wound-healing response to the implantation trauma and the mechanical properties of the implant as compared to the recipient tissue.

Finally, the resistance to degradation of the prosthesis and the toxicity of these degradation products is an important parameter [13].

Several techniques are available for changing the surface characteristics of biomaterials (see Table II). Physicochemical modification by the plasma-gas discharge technique for instance was used to change the surface properties of the stainless steel stent described in Chapter 3. Biological modifications are also frequently used such as heparin immobilization [18] and cell seeding (e.g. endothelium [19]).

Mechanical properties of the implant such as rigidity, elasticity and generation of (outward) force with concomittant diameter or length change of the device, can be varied by changing the composition of the basic material of the device or by changes in design (e.g. weaving angle of self-expanding devices). Heat treatments (heat setting) of the material can also influence the mechanical behaviour of a material.

Table II Summary of techniques available for the modification of surface characteristics. Modified from Hoffman, 1987[17]

Physicochemical modification:

- physical deposition of a coating (e.g. polyurethanes)
- chemical modification (e.g. PEO, -OH blocking)
- graft co-polymerization by radiation, (photo)chemical techniques (e.g. hydrogels)
- plasma gas discharge for etching or deposition (e.g. fluorocarbons)

Biological modification:

- pre adsorption of proteins (e.g. fibronectin, albumin)
- drug or enzyme immobilization (e.g. heparin, prostaglandins)
- cell seeding (endothelium)
- preclotting (fresh whole blood)

Testing of stents.

Several in vitro methods (Table III) are available for bench testing of biomaterials [20] and to assess their possible value as a vascular implant. These tests allow a selection of potentially suitable materials and designs. The composition of the material is important with respect to possible allergic reactions to metal components. Behaviour in cell culture is

important to determine cytotoxicity, leucocyte proliferation and activation, and mutagenicity.

Table III Summary of techniques for in vitro and in vivo testing of endovascular prostheses.

In Vitro:	- Surface characteristics (chemistry, charge, topography)
	- EDAX element analysis (Energy Dispersion Analysis of X-rays)
	- Mechanical behaviour (radial force, hysteresis)
	- Haemocompatibility (e.g. coagulation, platelet aggregation)
	- Cell culture (cytotoxicity, leucocyte activation test)
	- Mutagenicity / teratogenicity tests
Ex vivo:	- Extracorporeal circulation (arteriovenous shunts)
In Vivo:	- Intravascular (or subcutaneous) implantation
	- Acute and long term patency (quantitative angiography)
	- Histology (woundhealing, morphometry)
	- Transmission and scanning electron microscopy

The outermost layer however, is responsible for the haemo-compatibility profile, as this is the layer in contact with blood and tissue and can insulate the innermost components. Haemocomplatibility can be studied by testing platelet aggregation and clotting time of whole blood in the presence of the stent. Studying thrombogenicity under flowing blood conditions can be performed ex-vivo using extracorporeal (arteriovenous) shunts [21]. The mechanical behaviour of the prosthesis (deformation of the devices (creep, hysteresis), generation of radial force, it's ability to withstand compression) should be known before proceeding to implantation as these data are needed to ensure proper placement and unwanted migration of of the device.

Ultimately the behaviour of a material and its design should be tested in vivo (Table III) as the influence of vascular components cannot be ignored. Additionally, we will have to take into account the fact that the final target vessels are diseased atherosclerotic arteries and vein grafts of usually older patients. The thrombotic response to injury in these vessels will be more pronounced than in healthy arteries of the young pigs described in this thesis [22]. A model of accelerated arteriosclerosis in arteriovenous bypass grafts using overdistended veins as described in Chapter 5, is perhaps an alternative. In these narrowed grafts the thrombotic response to injury will be more pronounced, and will increase our understanding of the processes that take place in the clinical setting.

Scope of this Thesis.

In order to gain insight in the effects of stenting, we studied the process of wound healing and the short- and long-term effect of these permanently present foreign bodies. Both thrombogenic and less thrombogenic metals were evaluated with respect to thrombogenicity and tissue response. Synthetic polymers were evaluated with respect to improving the haemo-compatibility and tissue-compatibility profile of these devices.

Stenting of normal porcine arteries. In Chapter 2, a balloon-expandable tantalum stent is described, tested in normal porcine coronary arteries for one and four weeks, and an indication is given of the process of wound healing and of the extent of intimal hyperplasia in these arteries. In chapter 3 a self-expanding stainless steel stent was tested in normal porcine coronary arteries. Luminal change was assessed at one, four, and twelve weeks using quantitative angiography, while histologic analysis was performed at twelve weeks only. Additionally, the efficacy of a polymer coating as well as pharmacological treatment (anti-platelet and anti-coagulant treatment) aimed at reducing acute thrombotic complications and intimal hyperplasia was studied. In an attempt to improve some of the features of stents, early thrombogenicity and barotrauma, a polymeric stent was developed and tested in vitro and in vivo and the results are discussed in Chapter 4.

Stenting of vein grafts. Using a model of early vein graft narrowing in pigs, stenting was studied to assess the potential benefit of single and multiple stent implantation, compared to plain balloon angioplasty (Chapter 5). Stenting of both diseased and healthy arteries in animals of similar age and using the same stent, enables the assessment of behaviour of this stent under different circumstances. Pathologic examination of human saphenous vein bypass grafts treated with the self-expanding stainless steel stent (used in pigs in Chapter 3), is discussed in Chapters 6 and 7. This allowed for a comparison between the effect of stents when implanted in healthy porcine coronary arteries, porcine arteriovenous grafts and diseased human arteriovenous grafts.

Vascular (dys)function. To assess long-term effects of stenting, a physiological study was undertaken to determine vascular function (Chapter 8). Angiotensin metabolism in stented porcine coronary arteries was studied, as angiotensin II is implicated as a growth factor or as a modifier of growth response of smooth muscle cells.

Concluding remarks on these studies are presented in Chapter 9.

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Chapter 2

Coronary stenting with a new, radiopaque, balloon-expandable endoprosthesis in pigs.

Coronary Stenting With a New, Radiopaque, Balloon-Expandable Endoprosthesis in Pigs

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Background. **Intracoronary stents may be effective when used as "bail-out" devices for acute complications after percutaneous transluminal coronary angioplasty. Furthermore, preliminary reports have demonstrated some promising results with stents with regard to the reduction of restenosis. Several stent devices are available for preclinical and clinical evaluation. The use of these stainless-steel stents has been limited by poor visibility during fluoroscopy and thrombogenicity during the first days to weeks after implantation. We therefore investigated the immediate and short-term effects on arterial patency of a new, radiopaque, balloon-expandable coil stent in normal coronary arteries of pigs.**

Methods and Results. **In 10 animals, a stent was placed in two of the three epicardial coronary arteries. During the implantation procedure, the animals received heparin; after the procedure, no antithrombotic drugs were administered. After 1 week (five animals and 10 stents) or 4 weeks (five animals and 10 stents), repeat angiography was performed, followed by pressure-fixation of the coronary arteries for light and electron microscopic examination. Angiographic analysis revealed that all stented coronary segments were patent and without signs of intraluminal defects. Scanning electron microscopy showed complete endothelial covering of all stents within 7 days. Light microscopy showed a reduced tunica media locally under the stent wires, which resulted from exerted pressure. The neointima on top of the stent wires measured 56 μm (range, 42–88 μm) after 1 week and 139 μm (range, 84–250 μm) after 4 weeks.**

Conclusions. **Results from this study show that this radiopaque endoprosthesis can be safely placed in normal coronary arteries of pigs. After 4 weeks, all stents were patent and there was no need for additional antithrombotic treatment, whereas neointimal proliferation was limited.** (*Circulation* **1991;83:1788–1798**)

Percutaneous transluminal coronary angioplasty can be used to treat patients with atherosclerotic coronary artery disease and has a high initial success rate.[1,2] However, in 2–5% of cases, acute or subacute occlusion at the angioplasty site occurs.[3–4] Although the occluded artery can occasionally be successfully recanalated, it is frequently necessary to proceed to emergency coronary bypass graft surgery.[1,3] The efficacy of pharmacological therapy on acute complications is still undetermined, although the administration of aspirin appears to be an accepted approach.[5–9] Restenosis after successful coronary angioplasty remains, however, the second important factor that limits the efficacy of this procedure.[10,11] Pharmacological treatment to reduce the incidence of restenosis has so far been without effect,[12] although the addition of ω-3 fatty acids to standard antithrombotic drugs has shown some promise.[13] The implantation of vascular endoprostheses, attempted in the early days of angioplasty for the treatment of procedure-related complications,[14] may be useful in avoiding acute surgical intervention or preventing coronary restenosis.[15] Experimental and clinical experiences with these devices, however, indicate that their poor fluoroscopic visibility can make the implantation arduous. Furthermore, the thrombogenic nature of stainless-steel devices remains a concern, necessitating the administration of stringent anticoagulant therapy.[16,17] We therefore studied the short-term angiographic patency of a new, radiopaque, tantalum stent after implantation in the coronary circulation in pigs.

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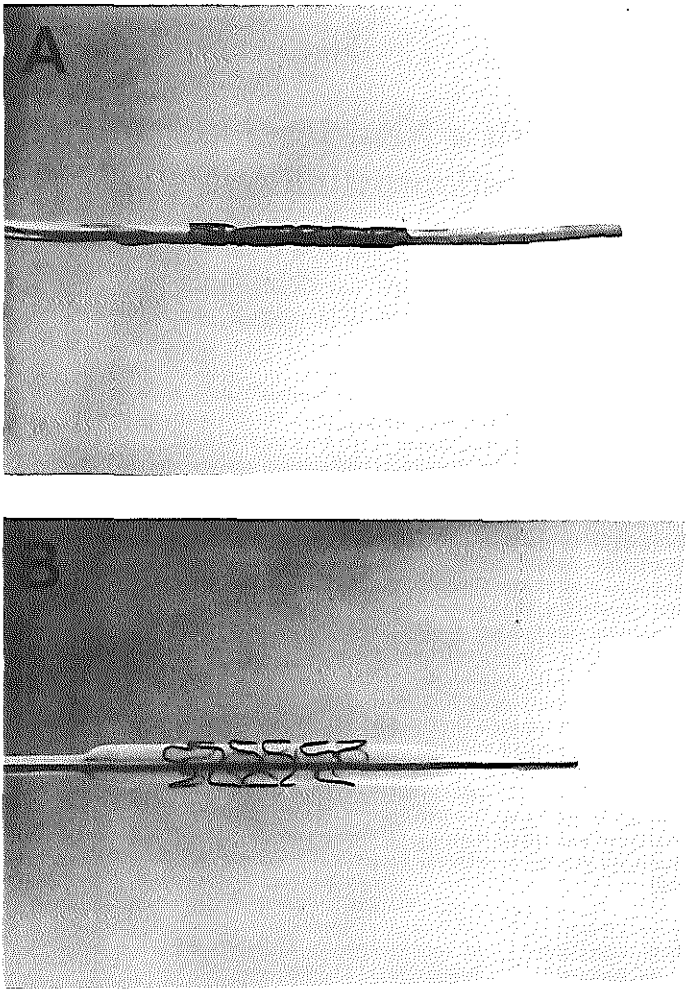

FIGURE 1. Panel A: *Photograph of Wiktor stent crimped on balloon of a standard balloon angioplasty catheter.* Panel B: *Photograph of Wiktor stent fully expanded by inflation of balloon to 8 atm.*

Methods

Balloon-Expandable Intracoronary Stent

The balloon-expandable stent used in the present study (Wiktor, Medtronic, Inc., Minneapolis, Minn.) is constructed of a single tantalum wire (0.127 mm diameter) formed into a sinusoidal wave and wrapped into a helical coil structure. This prosthesis is crimped onto the deflated polyethylene balloon of a standard angioplasty catheter (Figure 1A). By inflating the balloon, the diameter of the stent increases without alteration of its length (Figure 1B). The maximal diameter of the balloon after inflation determines the ultimate size of the prosthesis after implantation. One inflation at 8 atm is sufficient to open the stent and allows the safe withdrawal of the deflated balloon (Figure 2). The diameters of the balloons of the mounted angioplasty catheters used in the present study were 3.0 and 3.5 mm, and the lengths of the prostheses ranged from 14 to 16 mm. For the balloon catheters used with stents of these sizes, the crimped stent profile is approximately 1.5 mm. An advantage of the delivery system used is that after stent expansion, the balloon will rewrap tightly without excessive winging, which will facilitate balloon removal from the deployed stent. The manufacturer has indicated that although this device may be deliverable with a variety of catheters, the device will be marketed as a ready-to-use complete delivery system to ensure the safe delivery of the stent by controlling the difficult crimping process during manufacture.

Animal Preparation

Experiments were performed in Yorkshire pigs (weight, 40–46 kg; HVC, Hedel, The Netherlands).

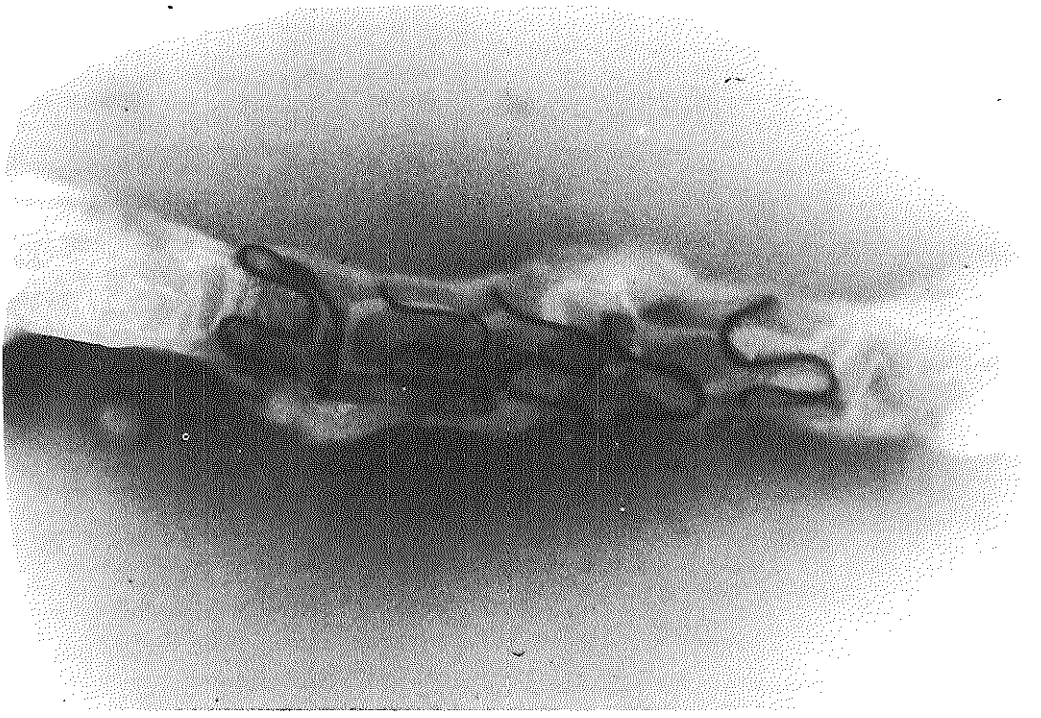

FIGURE 2. *Photograph of expanded Wiktor stent immediately after placement in a porcine coronary artery from which the adventitia has been removed.*

The investigations were performed according to the "Guide for the Care and Use of Laboratory Animals" (DHEW publication No. NIH-80-23, 1980), and the protocol was approved by the Committee on Experimental Animals of Erasmus University. After an overnight fast, the animals were sedated with 20 μg·kg ketamine hydrochloride. After endotracheal intubation, the pigs were connected to a ventilator that administered a mixture of oxygen and nitrous oxide (1:2 vol/vol). Anesthesia was maintained with 1–4 vol% enflurane, and pancuronium bromide was used as a muscle relaxant. Antibiotic prophylaxis was administered by an intramuscular injection of 1,000 mg of a mixture of procaine penicillin G and benzathine penicillin G.

Under sterile conditions, an arteriotomy of the left carotid artery was performed, and a 9F introduction sheath was placed. Next, 5,000 IU heparin sodium was administered, and an 8F guiding catheter was advanced to the ascending aorta. After measurement of arterial blood pressure and heart rate and withdrawal of an arterial blood sample for the measurement of blood gases and acid-base balance (settings of the ventilator were corrected if necessary), left and right coronary angiography was performed using iopamidol (Iopamiro 370, Dagra, Diemen, The Netherlands) as contrast agent. Eleven animals underwent the catheterization procedures. One animal was excluded from the study because angiography before stent implantation showed an occluded left coronary artery. At autopsy, air embolism of the coronary artery was demonstrated.

Stent Implantation

After angiography and with the diameter of the guiding catheter used as a reference, a segment with a diameter of 2.5 or 3.0 mm was selected in two of the three large coronary arteries (left anterior descending coronary artery, left circumflex coronary artery, and right coronary artery). No attempt was made to avoid side branches or angulated coronary segments. Then, a 3.0-mm (for 2.5-mm coronary segments) or 3.5-mm (for 3.0-mm coronary segments) balloon angioplasty catheter with a stent coil crimped on its deflated balloon was advanced over a 0.014-in. steerable guide wire to the site preselected for implantation. During all of our experiments, tracking and ease of placement in the coronary vasculature was good. After administration of an additional 2,500 IU heparin through the guiding catheter, the balloon was first inflated to a pressure of 8 atm for 30 seconds and then deflated, and negative pressure was maintained

TABLE 1. Stents and Implant Sites

Stent diameter (mm)	Stents (*n*)	Coronary artery sites	Sites (*n*)
3.0	10	RCA	1
		LAD	5
		LCx	4
3.5	10	RCA	4
		LAD	2
		LCx	4

RCA, right coronary artery; LAD, left anterior descending coronary artery; LCx, left circumflex coronary artery.

for 20 seconds. The angioplasty catheter was advanced slightly; if the marker in the middle of the balloon could be moved independent of the stent, the catheter was slowly withdrawn while leaving the stent in place. This implantation procedure was repeated in the second chosen coronary artery. (Implant sites are listed in Table 1). After repeat angiography of the stented coronary arteries, the guiding catheter and the introducer sheath were removed, the arteriotomy was repaired, and the skin was closed in two layers. The animals were allowed to recover from anesthesia; no postprocedure antithrombotic drugs were administered.

Follow-up Angiography

The catheterization procedure for follow-up angiography was identical to that described above. Coronary angiography was performed in the same projection as during implantation. Five animals (10 stents) were restudied after 1 week, whereas the other five animals (10 stents) were restudied after 4 weeks. Thereafter, the thorax was opened by a midsternal split, a lethal dose of sodium pentobarbital was injected intravenously, and immediate cross-clamping of the ascending aorta was performed. After puncturing the aortic root above the coronary ostia, 500 ml of saline followed by 400 ml of buffered glutaraldehyde was infused under a pressure of 120 mm Hg. Then, the heart was excised, and the coronary arteries were dissected from the epicardial surface. The stented segments and adjacent unstented segments were placed in 4% formaldehyde and 1% glutaraldehyde in phosphate buffer (pH 7.3) for at least 48 hours in preparation for microscopy.

Angiographic Analysis

Coronary angiograms (preimplantation, immediately after implantation, and after 1 or 4 weeks) were analyzed using the quantitative coronary angiography analysis system (CAAS).[18–20] Mean interpolated diameter at the site of stent placement was compared with the mean arterial diameter measured proximal and distal to the site of the stent.

Microscopic Examination

After fixation, the stent-containing arterial segments were divided lengthwise into two equal parts with a pair of fine scissors. The stent wires were removed from one half of each vessel. Both halves were washed in 0.1 M cacodylate buffer (pH 7.3), postfixed in 1% osmium tetroxide, and washed overnight in 0.1 M cacodylate buffer. The specimens were placed in 1% tannic acid for 60 minutes and 1% sodium sulfate for 10 minutes and again washed in 0.1 M cacodylate buffer. The vessel half containing the stent wires was dehydrated in graded ethanol series and critical point dried with liquid CO_2. Thereafter, it was mounted on a specimen table and sputtercoated with gold before examination in a scanning microscope (ISI-DS-130, Akashi Beam Technology, Tokyo, Japan). The other half of each vessel was dehydrated in graded acetone and embedded in epon. After sectioning and staining, microscopy was performed with a light microscope (BH2, Olympus, Tokyo, Japan) and an electron microscope (EM400, Philips, Eindhoven, The Netherlands). For measurement of the thicknesses of the various layers of the arterial wall, at least two sections of each stented coronary segment were selected. The sections were cut 90° transverse (as determined by the diameter of the stent wire) and projected onto a video screen, and the outer contours, external and internal elastic lamina, and endothelial lining were traced using an integrated image analysis system (IBAS-2000, Kontron, Oberkochen, FRG).[21] The distance between the endothelial lining and the internal elastic lamina was taken as the thickness of the intima.[22] The media was defined as the layer between the internal and the external elastic lamina.

Statistical Analysis

All data are expressed as mean±SEM unless otherwise stated. The significance of the changes in the angiographic data was evaluated by Duncan's new multiple-range test once an analysis of variance revealed that the samples represented different populations (random block design). The histological measurements were analyzed by the two-sample Wilcoxon test. A probability of less than 0.05 was considered statistically significant.

Results

Systemic Hemodynamics and Blood Gases During Implantation and Follow-up Angiography

During implantation and follow-up angiography, heart rates (89±6 and 92±7 beats/min, respectively), systolic arterial blood pressures (120±6 and 119±6 mm Hg), and diastolic arterial blood pressures (82±6 and 77±6 mm Hg) were comparable. The oxygenation of arterial blood and acid-base balance were also similar during stent placement and follow-up angiography and within the normal ranges (pH, 7.38±0.01; P_{O_2}, 144±9 mm Hg; P_{CO_2}, 43±1 mm Hg; base excess, 0.4±0.5 mmol/l).

Placement of Stent and Follow-up Angiography

A pilot study revealed that inflating the mounted balloon to as much as 6 atm was not sufficient to

ensure the safe withdrawal of the balloon after deflation. Furthermore, withdrawal of the catheter after a deflation period of less than 10 seconds could also disengage the device. We were able to avoid these situations by using a single inflation of 30 seconds to 8 atm followed by at least 20 seconds of deflation before the catheter was withdrawn. Special care proved to be necessary when inserting the device through hemostasis valves, guiding catheters, or coronary segments too narrow for the catheter profile to avoid dislodging the stent from the loaded balloon. Therefore, the position of the stent mounted on the balloon was inspected after passing the hemostasis valve and before leaving the guiding catheter.

In all 20 predetermined coronary segments (Table 1), a stent could be placed. Quantitative analysis of the angiograms confirmed that we chose to use mounted angioplasty catheters with 3.0-mm balloons (supplier specified) for stent placement in 2.5±0.1-mm artery diameters, that maximal inflated balloon diameters in these vessels were 3.0±0.1 mm (oversizing, 19±4%), and that the stented coronary segments after balloon deflation measured 2.6±0.1 mm (recoil, 9±5%). For the supplier-specified, 3.5-mm balloon catheters, these values were preimplantation, 2.7±0.2 mm; during maximal inflation, 3.2±0.1 mm (oversizing, 22±5%); and immediately after deflation, 2.9±0.1 mm (recoil, 9±4%). All animals survived the follow-up period. Repeat angiography revealed that all stented coronary arteries were patent after 1 and 4 weeks (Figure 3). Quantitative angiography showed that the mean diameters of the stented coronary segments after 1 and 4 weeks follow-up showed no statistically significant changes compared with the diameters immediately after placement (Figure 4). The diameter stenoses of the stent-containing coronary segments relative to the reference diameters proximal and distal to the stents measured 8±2% immediately after stent implantation, 15±4% at 1 week, and 17±4% at 4 weeks.

Light Microscopic Measurements

The stent wires were incorporated in the arterial wall as early as 1 week after implantation. Measurement of the several arterial layers showed that the neointima covering the wires had a median thickness of 56 μm (range, 42–88 μm) after 1 week and increased to 139 μm (range, 84–250 μm) after 4 weeks (Figure 5). The neointima in the open or nonstrut areas of the stented segments had a median thickness of 25 μm (range, 9–38 μm) after 1 week and increased to 48 μm (range, 6–120 μm) after 4 weeks. The arterial media was considerably compressed under the stent wire. It was observed that in several vessels, the internal elastic lamina was disrupted at the wire site.

Light and Transmission Electron Microscopy

During the first week after stent implantation, neointimal hyperplasia was confined to the area in direct contact with the stent wires and consisted mainly of organized thrombi with a few layers of smooth muscle cells under the endothelial lining (Figure 6A).

After 4 weeks, neointimal hyperplasia completely covered the stented coronary segments. A few foam cells and some erythrocytes were found in the neointima as a trace of the thrombotic event that took place at the time of implantation. The bulk of the neointima comprised smooth muscle cells with their typical organization, changing from a longitudinal to a circumferential orientation, beginning at the luminal side of the vessel (Figure 6B).

No inflammatory or foreign body reaction was observed at 1 or 4 weeks, based on the absence of giant cells or infiltrative changes in either layer of the vessel wall.

Scanning Electron Microscopy

Scanning electron microscopy showed that at 1 week after placement of the stents, all wires were completely covered with endothelium (Figure 7A). The coronary arteries, however, showed an undulated luminal surface. After 4 weeks, the stent wires were barely detectable under the neointimal surface (Figure 7B). Fine corrugation of this surface could be demonstrated, although adherent thrombi or platelet aggregates were not observed.

Discussion

Radiopacity

Vascular endoluminal prostheses (stents) are undergoing clinical evaluation for their usefulness as scaffolding devices for acute occlusion after coronary angioplasty.[15,16,23,24] The efficacy of these devices has also been studied in the prevention and treatment of restenosis in aortocoronary bypass grafts and the native coronary circulation.[15,25–28] Although the long-term effect of stents used for these indications is still not determined, immediate angiographic results are excellent, and there is improvement during the first 24 hours.[29] However, the poor visibility of thin-wired, stainless-steel devices during fluoroscopy is a disadvantage during implantation. Both the inability to place stainless-steel, balloon-expandable stents at preselected arterial sites and systemic embolization have been reported experimentally and clinically.[30–32] Furthermore, uncertainty about the precise placement of nonradiopaque stents will complicate the determination of immediate efficacy and late restenosis in the coronary segments in which the stent purportedly was placed. The stent used in the present study has the distinct advantage of being clearly visible under fluoroscopy (Figure 3), thus facilitating its safe placement.[33] With this device, there can be no discussion about which coronary segment is covered by it and which is not. Quantitative angiographic assessment is simplified, but not all problems have been solved; preliminary (unpublished) data from our laboratory show that videoden-

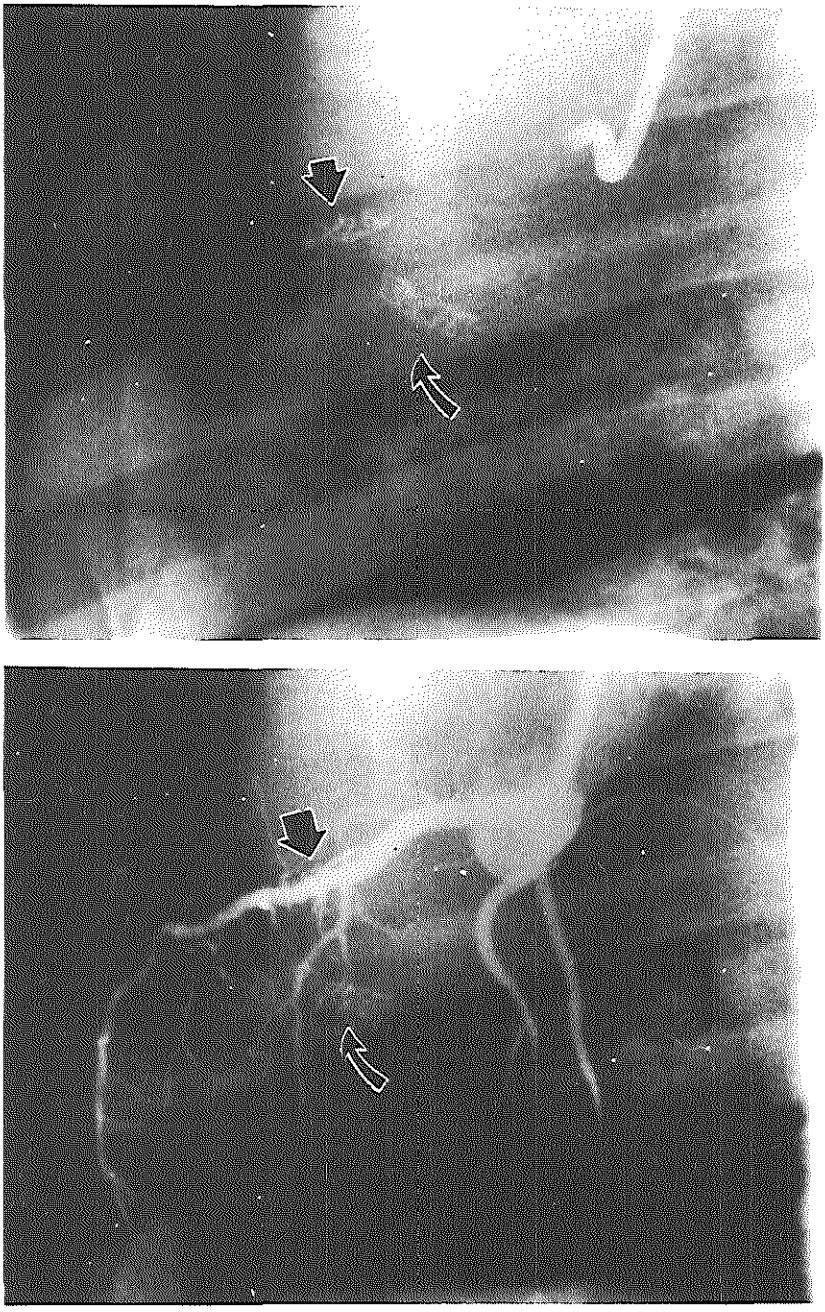

FIGURE 3. Top panel: *Nominal 3.0-mm-diameter Wiktor stent placed 4 weeks previous in left anterior descending coronary artery (large arrow) and nominal 3.5-mm-diameter Wiktor stent placed in right coronary artery (small arrow) clearly visible during radiography.* Bottom panel: *Coronary angiogram in left inferior oblique projection showing patent left anterior descending coronary artery without intraluminal defects associated with stent (large arrow).*

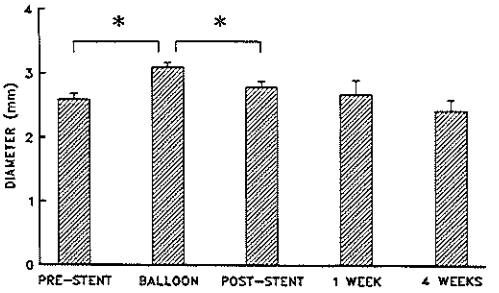

FIGURE 4. *Bar graph of quantitative angiographic assessment of stented coronary segments. Data are expressed as mean±SEM and represent pooled data of both balloon sizes. PRE-STENT, coronary segment immediately before stenting (n=20); BALLOON, balloon diameter during maximal inflation to 8 atm during placement of stent (n=19); POST-STENT, coronary segment immediately after stent placement (n=20); 1 WEEK, measurements of five animals (nine stents) 1 week after stent placement; 4 WEEKS, measurements of five animals (10 stents) 4 weeks after stent placement.* *p<0.05 *versus balloon.*

sitometric assessment will be influenced by the presence of any type of stent.

Thrombogenicity

Another major problem with stainless-steel stents is that they require elaborate anticoagulation therapy after implantation because of their thrombogenicity, which will increase the risk of complications associated with a hemorrhagic diathesis. In the present study, all stented coronary arterial segments were angiographically patent after 1 and 4 weeks without any signs of intraluminal defects, with antithrombotic drugs not administered during follow-up. Although we cannot exclude that intercurrent thrombi or platelet aggregates were formed during the first few days after implantation, stent occlusion did not occur; after 1 week, scanning microscopy showed a clean endothelial surface covering the stents. If thrombotic material of considerable size had embolized before that time, deaths would have occurred because the risk of lethal arrhythmias after coronary occlusion is very high in this species. That all animals survived may serve as circumstantial evidence against the occurrence of embolization of thrombus material. Thrombotic events associated with the stent have been reported experimentally in as many as 40% of study groups using other stents but can be reduced by choosing appropriate antithrombotic regimens.[30,31,34,35] The nonthrombogenic feature of this stent is not determined, but several factors may be involved. First, the metal itself (tantalum) may be a major determinant. The wire surface of any stent may undergo oxidation after implantation, and the resultant oxide, in this case, tantalum oxide, is not only very stable (implying fewer changes in surface charge) but also corrosion resistant.[36] Second, there is a large area of open space (91.6% and 92.3% for 3.0- and 3.5-mm expanded diameters, respectively) between the wires, resulting in less metal per surface area. Third, the entire stent surface is smooth. Finally, there is early endothelial covering (after 1 week, the Wiktor stents were completely covered by an endothelium-covered neointima).

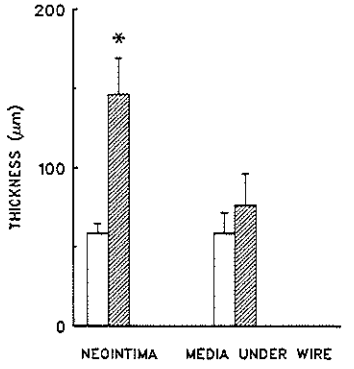

FIGURE 5. *Bar graph of thickness of neointima above stent wires and media under wires 1 week (open columns) and 4 weeks (striped columns) after stent implantation. Data are expressed as mean±SEM (n=7).* *p<0.05 *versus 1 week.*

Neointimal Hyperplasia

At 1 week after implantation, the luminal surface of the artery showed an undulated appearance. This may cause local turbulence in blood flow and fluctuations in wall shear stress, which may be factors determining endothelial cell turnover and intimal hyperplasia.[37–40] We could demonstrate that the thickness of the neointimal layer had considerably increased between 1 and 4 weeks after implantation. After 4 weeks, the luminal surfaces of the stented coronary segments were smooth. Future studies are necessary to determine whether the increase in intimal thickness levels off after 4 weeks. However, the median thickness of the neointima in the present study was not different from that reported for other types of stents.[30,31,41] Schwartz et al[42] emphasized that rupture of the internal elastic membrane after stenting may form a trigger for accelerated neointimal hyperplasia, but we observed considerable damage to this elastic lamina in several cases without significant restenosis (e.g., see Figure 6B). Other factors, such as considerable oversizing of the prosthesis with resultant damage to the arterial media, are probably involved.

Elastic Recoil

A potential beneficial effect of self-expanding stents is the prevention of elastic recoil after coronary angioplasty.[29] The balloon-expandable Wiktor stent lacks this effect, perhaps because of its passive radial force and relatively large area of open space. Quantitative angiographic assessment showed a recoil of 9% immediately after placement of the stent (Figure 4). Data

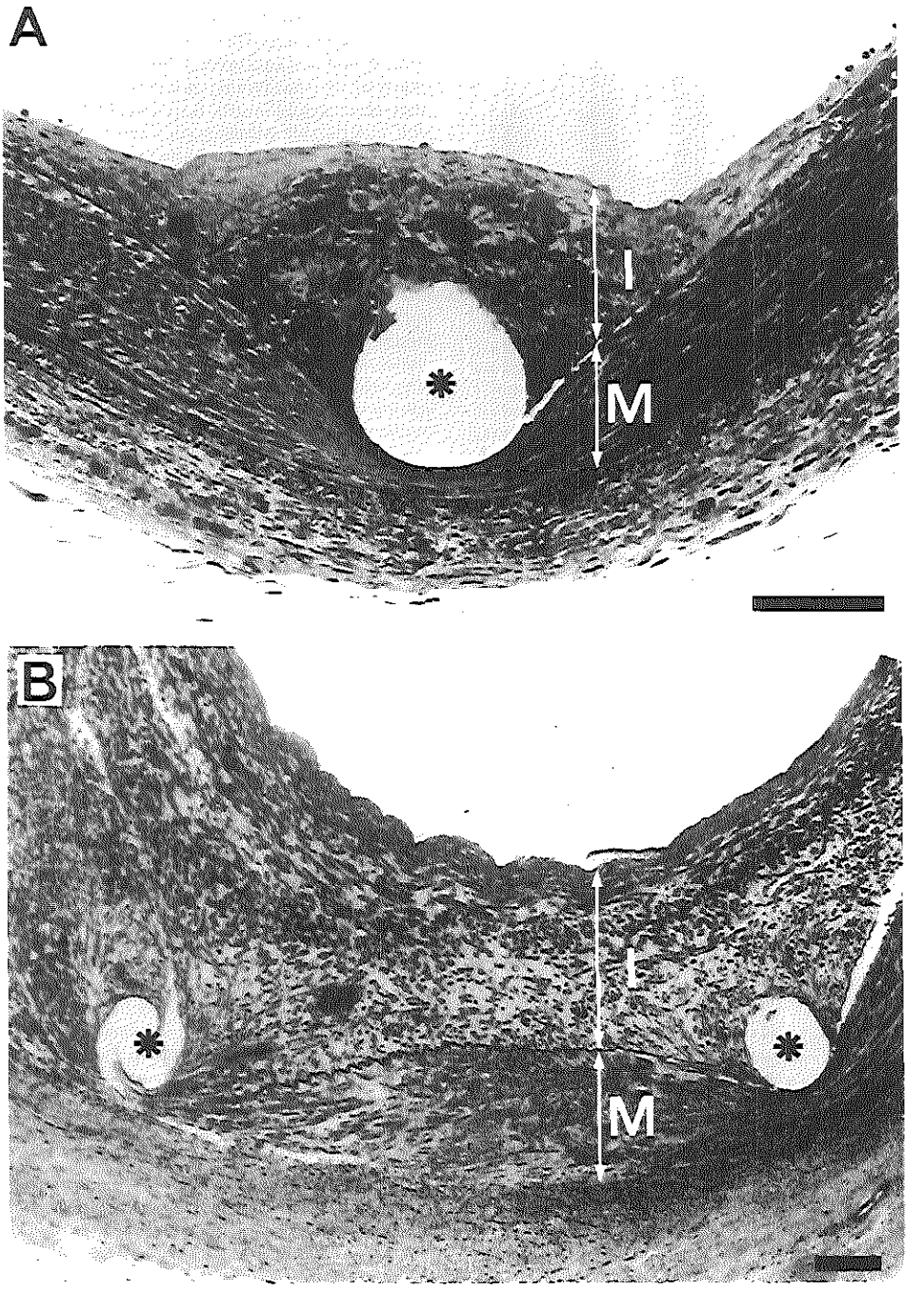

FIGURE 6. Panel A: *Photomicrograph of transverse section of left anterior descending coronary artery 1 week after placement of a Wiktor stent.* Panel B: *Photomicrograph of transverse section of left circumflex coronary artery 4 weeks after stent implantation (toluidine blue stain). I, intima; M, media. *Void formerly occupied by stent wire. Bar, 100 μm.*

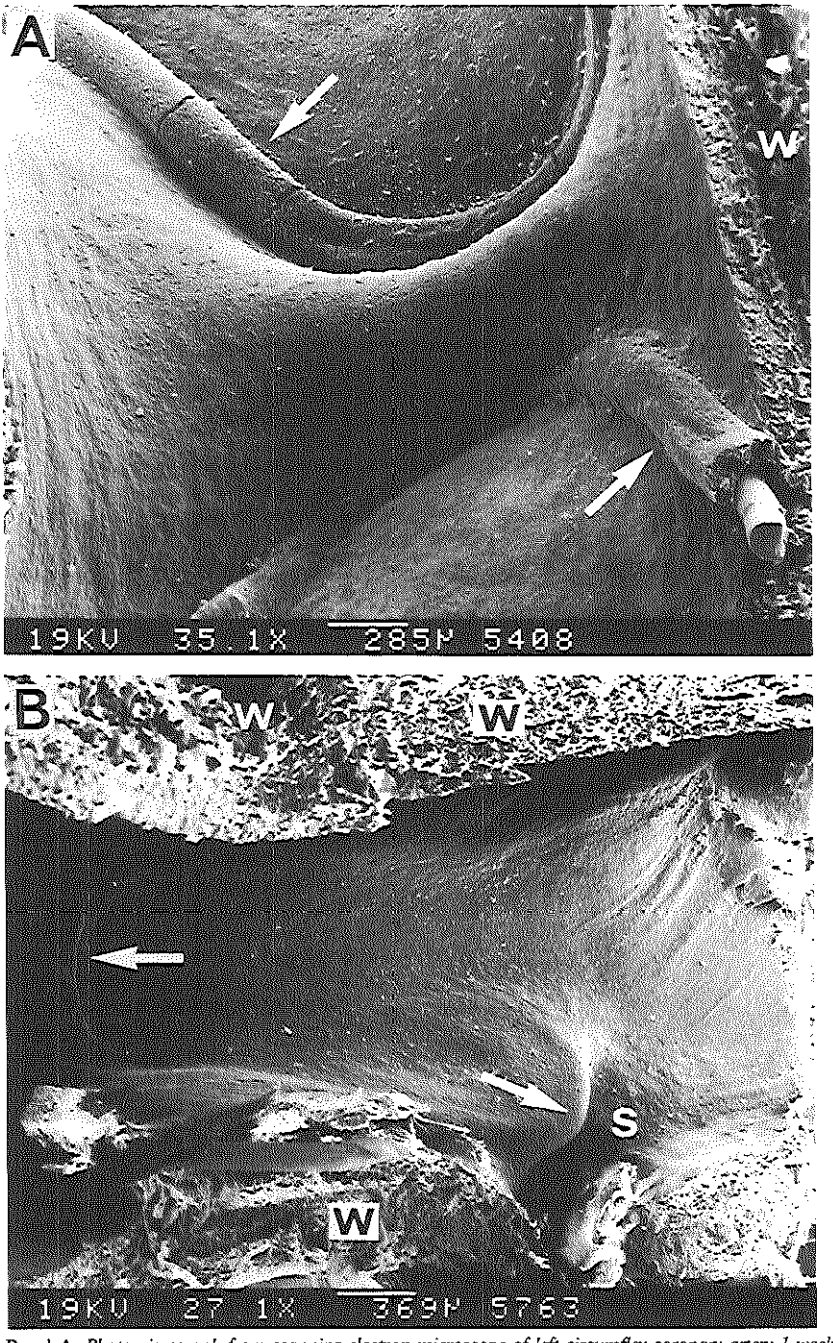

FIGURE 7. Panel A: *Photomicrograph from scanning electron microscope of left circumflex coronary artery 1 week after stent placement. Two parts of endothelium-covered stent wire coil (arrows) can be seen. Between the coils, part of the vessel wall (W) is bulging out.* Panel B: *Photomicrograph from scanning electron microscope of right coronary artery 4 weeks after stent placement. Under a layer of neointimal hyperplasia, the stent wire coil (arrows) is hardly visible. An embedded stent wire partly overhangs a side branch (S). W, cut face of vessel wall.*

from other balloon-expandable stents are not available or inconclusive. In vitro studies using another type of balloon-expandable tantalum stent also point toward considerable recoil.[43] However, which feature (metal or design) determines the elastic properties of a stent remains unknown.

Conclusion

Interpretation of the data obtained in the present study must be made with consideration that it involves the evaluation of a new device in normal coronary arteries of experimental animals. However, comparison of the results of the present study with data obtained for other stent devices in healthy experimental animals indicates that the Wiktor stent is less thrombogenic.[30,31,35] Whether this will also be true in atherosclerotic coronary arteries in swine, in which at least one of the other stent devices showed a low complication rate,[44] remains to be demonstrated. Also, the clear visibility of the Wiktor stent under fluoroscopy is a distinct advantage.

Acknowledgments

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KEY WORDS • percutaneous transluminal coronary angioplasty • stents • prostheses • electron microscopy

Chapter 3

Coronary stenting with polymer-coated and uncoated endoprosthesis in pigs

Coronary stenting with polymer-coated and uncoated self-expanding endoprostheses in pigs

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Background: Preliminary clinical reports suggest that metallic stents are thrombogenic during the first days to weeks after implantation but that restenosis rates after this period may be reduced.

Methods: We investigated the angiographic patency and histologically measured neointimal hyperplasia of 48 uncoated self-expanding, stainless steel mesh stents (Wallstent, Schneider [Europe] AG, Bülach, Switzerland) placed in normal coronary arteries of three groups of eight pigs that received postprocedure aspirin (ASA, group B), acenocoumarol (group C), or no medication (group A). The results were compared with those obtained in a fourth group of eight pigs (group D) receiving 15 polymer-coated (BioGold, PlasmaCarb Inc., Bedford, NH, USA) Wallstent stents, which are designed to reduce thrombogenicity without additional medication. After 1, 4, and 12 weeks, angiography was repeated. After the last angiogram the coronary arteries were pressure-fixated followed by light microscopy, immunocytochemistry, and electron microscopic examination.

Results: Group A showed a 38% thrombotic occlusion rate within 1 week, which was prevented by acenocoumarol or polymer coating, but not by ASA. Histologic analysis showed no difference in the thickness of neointimal hyperplasia between the groups (median values: 134, 101, 143 and 113 μm for groups A, B, C, and D, respectively). Polymer coating did not induce an inflammatory reaction.

Conclusions: Acenocoumarol and the polymer coating protected against early thrombotic occlusion of stainless steel self-expanding stents, but neointimal hyperplasia was not affected by preventive measures against stent thrombosis.

Coronary Artery Disease 1992, 3:631–640

Keywords: coronary angioplasty, grafts and prostheses, quantitative angiography, histology, immunocytochemistry, stents, antithrombotic treatment, pigs

Percutaneous transluminal coronary angioplasty (PTCA) is an accepted and increasingly used treatment for obstructive atherosclerotic coronary artery disease. Its initial success rate is high [1,2], but in 2% to 7% of cases, acute or subacute occlusion at the site of angioplasty occurs [3–5]. Except for the administration of aspirin [6–8], pharmacologic therapy has been ineffective, and, although redilatation of an acutely occluded artery can be successful, emergency coronary bypass surgery is frequently necessary [5]. The second important limitation of PTCA is late restenosis [9–11], which also can not be prevented by pharmacologic interventions [12–14].

A novel approach for the treatment of acute complications or the prevention of restenosis after PTCA may be the placement of an endovascular prosthesis (stent) [15–17]. Results from in vitro investigations [18], as well as from uncontrolled clinical studies with stainless steel stents in the coronary circulation indicate, however, that these devices are prone to acute or subacute thrombotic occlusion and do not

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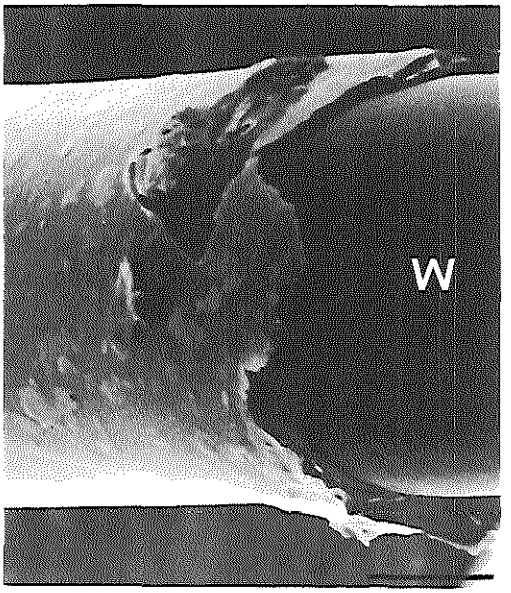

Fig. 1. Scanning electron micrograph of a stent wire, showing the polymer coating (Biogold, PlasmaCarb Inc., Bedford, NH) on the left, and the bare wire (W) on the right. *Bar* represents 25 μm.

eliminate, although they do reduce the occurrence of restenosis [19–24]. Coronary stents composed of tantalum are less thrombogenic than stainless steel stents in normal porcine coronary arteries [25], but the initial results in patients do not show this to be a detectable advantage [26]. Several approaches have been proposed to improve the surface properties of vascular prostheses [27]. One of the possibilities is the use of nonthrombogenic polymers as an outer coating on metal devices. Such a polymer coating has been applied to a self-expanding stainless steel stent to reduce its thrombogenic profile [28]. In the present study we compared the angiographic patency and histologic features of both polymer coated and not coated stents after implantation in the coronary circulation in pigs.

Methods

Self-expanding stainless steel stent

The endovascular prosthesis studied (Wallstent, Schneider [Europe] AG, Bülach, Switzerland) is a stainless steel, cylindric, open-weave wire mesh. The wires are 80 μm in thickness. The elastic properties of this prosthesis are such that its diameter can be substantially reduced by elongation. It can thus be constrained on a small-diameter delivery-catheter (diameter catheter plus stent is approximately 1.6 mm) that consists of two coaxial catheters. The proximal regions of the two coaxial catheters are joined by an invaginated rolling membrane, which effectively retains the prosthesis. Withdrawal of the outer catheter rolls back the membrane progressively, thus releasing the stent, which tends to return to its original diameter, thereby anchoring itself against the arterial wall. The unconstrained diameters of the stents used in this study were 3.0 and 3.5 mm, with a length of 15 to 19 mm.

Polymer coating of metallic stents

The polymer coating applied to the Wallstent has been referred to as the BioGold (PlasmaCarb Inc., Bedford, NH) coating and consists of a 30-nm thin layer of cross-linked methane (Fig. 1). A related coating consisting of acetylene/H_2/N_2 with a thickness of 20 nm on poly(methyl methacrylate) contact lenses showed good biocompatibility in rabbits and tissue culture [29]. The blood compatibility testing on BioGold-coated stents has been described in the patent application [28]. Blood compatibility testing with related coatings has been performed *in vitro* [30] and in connection with vascular grafts (*eg*, Dacron, Teflon, [Dupont, Wilmington, DE], and Gore-

Table 1. Comparison of study groups according to number of implanted stents and antithrombotic treatment

Study group	Animals, *n*	Stent type	Stents, *n*	Diameter of stents, *mm*	Antithrombotic treatment	Duration of treatment, *d*
Group A	8	Uncoated self-expanding stainless steel mesh stent (Wallstent, Schneider [Europe] AG, Bülach, Switzerland)	16	3, 3.5	—	NA
Group B	8	Uncoated Wallstent	16	3, 3.5	aspirin (100 mg/d)	11*
Group C	8	Uncoated Wallstent	16	3, 3.5	coumarin†	9‡
Group D	8	Polymer-coated (Biogold, PlasmaCarb Inc., Bedford, NH, USA) Wallstent	15	3.5	—	NA

*Aspirin doseage was begun 3 days before stent implantation and continued till 7 days after implantation.
†The dose of coumarin was adjusted according to the thrombo test.
‡Coumarin was begun 1 day before stent implantation and continued till 7 days after implantation.

Table 2. Use of stents in three coronary arteries

Study group*	LAD, *n*	LCX, *n*	RCA, *n*
Group A			
3.0-mm stent	4	2	2
3.5-mm stent	2	3	3
Group B			
3.0-mm stent	4	2	1
3.5-mm stent	2	2	5
Group C			
3.0-mm stent	2	2	2
3.5-mm stent	4	3	3
Group D			
3.5-mm stent	7	4	4

*Dimension of stent diameter given.
LAD—left descending anterior; LCX—left circumflex; RCA—right coronary artery.

Tex [Gore and Associates, Inc., WL, Elkton, MD]) in a nonhuman primate, vascular shunt model [31,32]. The thrombo-resistant features of the BioGold coating may be explained by its hydrophilic surface and reduced adsorption of plasma proteins, resulting *in vitro* in the prolongation of the coagulation test and *in vivo* in enhanced graft patency.

Animal preparation

Experiments were performed in Yorkshire pigs (40–46 kg; HVC, Hedel, the Netherlands). The investigations complied to the *Guide for the Care and Use of Laboratory Animals* [33] and the protocol was approved by the Committee on Experimental Animals of the Erasmus University. After an overnight fast, the animals were sedated with 20 $mg.kg^{-1}$ ketamine hydrochloride. Following endotracheal intubation, the pigs were connected to a ventilator that administered a mixture of oxygen and nitrous oxide (1:2, v/v). Anesthesia was maintained with 1 to 4 vol% enflurane, while pancuronium bromide was used as a muscle relaxant. Antibiotic prophylaxis was administered by an intramuscular injection of 1000 mg of a mixture of procaine penicillin-G and benzathine penicillin-G.

Under sterile conditions an arteriotomy of the left carotid artery was performed, and a 9F introduction sheath was placed. Then 5000 IU of heparin sodium was administered and an 8F guiding catheter was advanced to the ascending aorta. After measurement of arterial blood pressure and heart rate and withdrawal of an arterial blood sample for the measurement of blood gases and acid-base balance (settings of the ventilator corrected, if necessary), left and right coronary angiography was performed using iopamidol (Iopamiro 360, Dagra BV, Diemen, the Netherlands) as contrast agent. Thirty-two animals underwent the catheterization procedures. The animals were divided in four study groups. Three groups of eight animals each received two Wallstent stents and in two of these three groups antithrombotic prophylaxis was administered during the first study week as summarized in Table 1. The fourth group of eight animals received the polymer-coated Wallstent. In this group additional antithrombotic agents were not used.

Stent implantation

From the angiograms, and using the diameter of the guiding catheter as a reference, a segment with a diameter of 2.5 or 3.0 mm was selected without attempting to avoid side branches or curved coronary segments in two of the three epicardial coronary arteries (left anterior descending coronary artery [LADCA], left circumflex coronary artery [LCXCA], right coronary artery [RCA]). Thereafter, a 3.0 mm (for 2.5-mm coronary segments) or a 3.5 mm (for 3.0-mm coronary segments) stent, constrained on its delivery catheter, was advanced over a long guide wire to the site selected for implantation. Of the uncoated Wallstents, nominal unconstrained stents with a diameter of 3.0 mm and 3.5 mm were used, whereas of the polymer-coated Wallstents, only diameters of 3.5 mm were available for this study. After administration of additional 2500 IU of heparin through the guiding catheter, the stent was released. This implantation procedure was repeated in the second selected coronary artery. All implant sites are listed in Table 2. After repeat angiography of the stented coronary arteries, the guiding catheter and the introducer sheath were removed, the arteriotomy repaired, and the skin closed in two layers. The animals were allowed to recover from anesthesia, and antithrombotic prophylaxis was administered as summarized in Table 1. The coumarin dosage was adjusted to obtain a thrombotest of more than 180 seconds (usually 2 mg per day), and the ASA dosage was 100 mg per day. Both drugs were administered orally.

Hematologic measurements

Immediately before implantation and again after 1 week, thrombotest was performed in the animals of groups A, B, and C. In some of the animals treated with coumarin this measurement was repeated at 4 weeks to assess whether the coagulation parameter had returned to baseline.

Follow-up angiography

The catheterization procedure for follow-up angiography was identical to that described above. Coronary angiography was performed in the same projection, and with

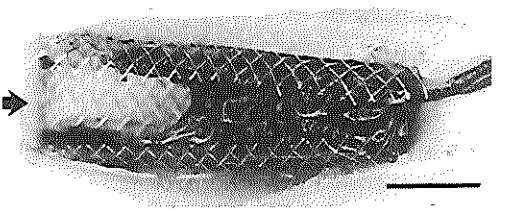

Fig. 2. Macroscopic photograph of a stent, which showed thrombotic occlusion 26 hours after placement in a right coronary artery (group A). The *arrow* indicates the blood flow direction. *Bar* represents 5 mm.

Table 3. Percentage of stented coronary arteries that were patent at angiographic follow-up at 1, 4, and 12 weeks after implantation

	Patency, %		
Study group	1 wk	4 wk	12 wk
Group A	62*	56*	56*
Group B	82	75†	75†
Group C	100	88	88
Group D	100	100	100

*$P<0.05$ vs groups C and D.
†$P<0.05$ vs group D.

identical geometry of the radiographic equipment as during implantation. Animals were restudied after 1, 4, and 12 weeks. After the last procedure the thorax was opened by a mid-sternal split and a lethal dose of sodium pentobarbital was injected intravenously, immediately followed by cross-clamping of the ascending aorta. After puncturing the aortic root above the coronary ostia, 500 mL of saline followed by 400 mL of buffered fixative was infused under a pressure of 120 mm Hg. Then the heart was excised and the coronary arteries were dissected from the epicardial surface. The stented coronary segments and adjacent not-stented segments were placed in 4% paraformaldehyde and 1% glutaraldehyde in phosphate buffer (pH, 7.3) for at least 48 hours in preparation for electron microscopy, or in 4% paraformaldehyde in phosphate buffer (pH, 7.3) for at least 24 hours in preparation for light microscopy and immunocytochemistry.

Endothelial integrity

After the last angiogram 500 mL of 0.3% Evans Blue in saline (filtered through an 0.2-μm pore filter) was infused in three animals for the macroscopic assessment of the integrity of the endothelial lining using the dye exclusion technique [34]. After washing out the excess dye with an additional 500 mL of saline, buffered fixative was infused as described in the previous section.

Quantitative angiographic analysis

Coronary angiograms (pre-implantation; immediately after implantation; and after 1, 4, and 12 weeks) were analyzed using the quantitative coronary angiography analysis system (CAAS) [35]. Mean luminal diameter at the site of stent placement was measured.

Microscopic examination

After fixation, the specimens were processed for light- and electron microscopic examination as described earlier [25]. Qualitative low-power examination was performed for all eight vessels after staining with hematoxylin-azophloxin and resorcin-fuchsine, an elastin stain, using a light microscope (BH2, Olympus, Tokyo, Japan).

For the measurement of the thickness of the various layers of the arterial wall, at least four perpendicular resorcin-fuchsine stained sections of the proximal and distal part of each stented arterial segment were projected on a video screen and the external and internal elastic lamina and the endothelial lining were traced using an integrated image analysis system (IBAS-2000, Kontron, Oberkochen, FRG) [36]. The distance between the endothelial lining and the internal elastic lamina was taken as the thickness of the intima. The media was defined as the layer between the internal and external elastic lamina. Scanning electron microscopy (using a JSM 25, Jeol Ltd., Tokyo, Japan) was performed using earlier described methods [25].

Statistical analysis

All data are expressed as mean ± SEM. The significance of the changes in the quantitative angiographic data were evaluated by Kruskal-Wallis analysis of variance (ANOVA) using a statistical graphics system (Statgraphics, STSC Inc., Rockville, MA). The qualitative angiographic data were analyzed using the chi-square test. The histologic measurements were analyzed by the Wilcoxon rank-

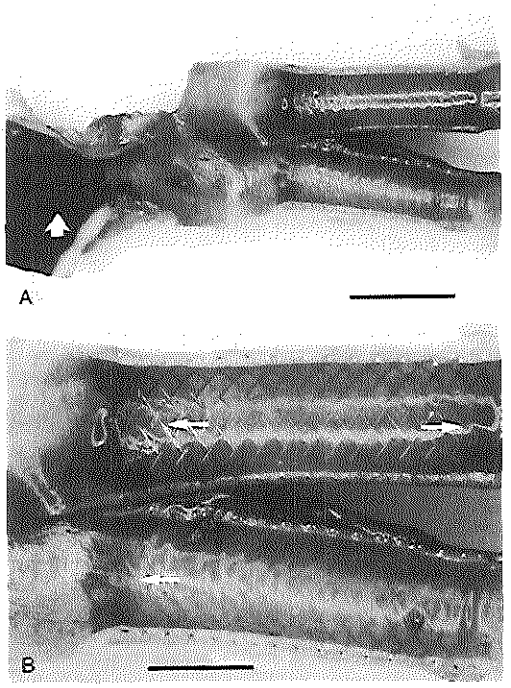

Fig. 3. A, Macroscopic photograph of a left circumflex coronary artery (group B) 12 weeks after stent implantation. The Evans Blue dye exclusion test showed an intensely stained coronary ostium (*arrow*). *Bar* represents 10 mm. **B**, Detail of the stented segment of figure 3A, showing a slightly darker rim (*arrows*) within both extremities of the stent, indicating a loss of integrity of the endothelial lining. *Bar* represents 5 mm.

sum test. A P value less than 0.05 was considered statistically significant.

Results

Systemic hemodynamics and arterial blood gases during implantation

During implantation heart rate (106±3 bpm^{-1}), systolic arterial blood pressure (123±4 mm Hg) and diastolic arterial blood pressure (89±3 mm Hg) were similar for all the experimental groups. The oxygenation of arterial blood and acid-base balance were also similar and within the normal range (pH, 7.36±0.02; Po_2; 139±9 mm Hg; Pco_2, 43±2 mm Hg).

Hematologic measurements

Immediately before implantation of the stents, the thrombotest values were normal in the untreated (24±1 s) and in the aspirin-treated animals (25±1 s) and did not change during the follow-up period. The animals treated with acenocoumarol showed already a significant prolongation of TT before the implantation procedure (64±19 s, $P<0.05$) that was sustained during the first week after placement of the stents (134±29 s, $P<0.05$) but had returned to normal values after 4 weeks (24±1 s).

Placement of stents and follow-up angiography

In all 63 predetermined coronary segments (Table 2) a stent could be placed. In group A only 62% of the stents were patent at 1 week (Table 3). In this group three animals died suddenly at 20, 24, and 26 hours after the implantation procedure. Immediate autopsy showed a platelet-rich occlusive thrombus in all stented coronary segments of these animals (Fig. 2). In one additional animal in this group, one of the two stent-containing arteries occluded between 1 and 4 weeks but did not cause adverse events. In group B three animals also died suddenly, at 12 hours, 2 days, and 14 days after the procedure. Four of the six stented coronary arteries proved to be occluded by thrombus at autopsy. In group C, one animal died suddenly after 9 days (2 days after stopping acenocoumarol treatment; TT at 7 days was >200 s, <5 %). Autopsy revealed that both stented coronary arteries were blocked by thrombosis, resulting in a circumferential left ventricular infarction and pulmonary edema. In group D, complications did not occur and all coronary arteries proved to be patent at 12 weeks. The angiographic patency rate in the groups C and D was significantly higher than that observed in group A ($P<0.05$), but only the patency rate of the coated group, D, was significantly higher compared with group B at 4 and 12 weeks.

Quantitative analysis of the angiograms showed no differences in the baseline diameters of the coronary arteries between the groups (Table 4). There was no significant increase in diameter in either group after stent implantation. No significant changes occurred between the groups during follow-up.

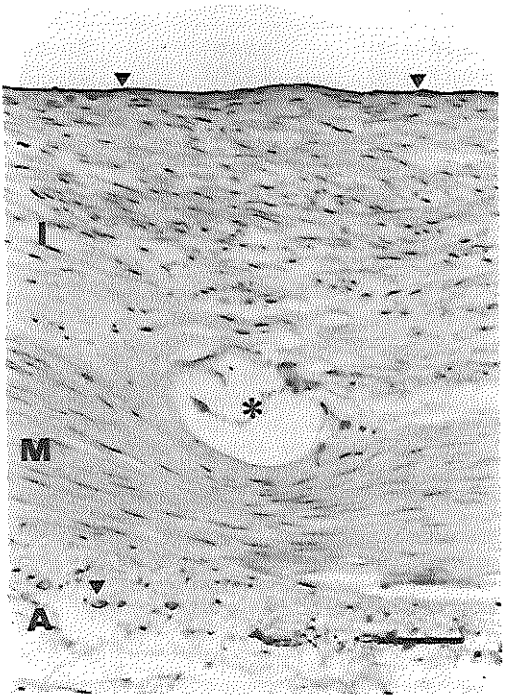

Fig. 4. A positive identification of endothelium (*arrows*) with the horse-radish peroxidase labeled lectin Bandeirea Simplicifolia [48] is shown as dark-brown as seen at the luminal aspect of this coronary artery 12 weeks after stenting. Also the capillary endothelium (*arrow*) in the adventitia (A) exhibits positive staining. *Asterisk* indicates stentwire void. *Bar* represents 100 μm. I—intima; M—media.

Endothelial integrity

In the three animals in which the integrity of the endothelial lining was assessed with the Evans Blue dye exclusion test, macroscopic examination revealed a slightly darker rim within the extremities of the stents in all arteries (Fig. 3), indicating enhanced passive permeability of the endothelial layer at these sites. The covering of the body of the stents was stained locally and was most pronounced at the origin of side branches. The coronary ostia, however, were most intensely stained by Evans Blue, as

Table 4. Mean luminal diameter of the stented coronary segments as determined by quantitative angiographic analysis before and after stent implantation

	Mean luminal diameter, *mm**				
		Post stent			
Study group	pre stent	Immediately	1 wk	4 wk	12 wk
Group A ($n=7$)	3.3±0.1	3.0±0.3	3.1±0.2	3.3±0.3	3.8±0.2
Group B ($n=6$)	2.6±0.1	2.8±0.3	3.0±0.2	3.0±0.2	3.0±0.2
Group C ($n=7$)	3.0±0.3	3.1±0.2	3.0±0.2	2.9±0.3	3.3±0.2
Group D ($n=7$)	2.8±0.1	3.0±0.2	2.9±0.2	2.8±0.2	2.9±0.2

*Values are mean±SEM.

a marker of the damage by the catheter tip during angiography.

Light microscopic measurements

Measurement of the average neointimal thickness on top of the stent wires (Table 5) showed no differences between the groups. The range of values was, however, considerably wider in groups A (range, 39 to 413 μm) and C (range, 40 to 239 μm, with one additional artery averaging 756 μm) than in groups B (range, 73 to 192 μm) and D (range, 44 to 167 μm).

The arterial media was compressed underneath the stent wire, illustrated by a mean thickness of about 80 μm under the stent wires, compared with an average thickness of 120 μm between the wires.

Light and transmission electron microscopy and immunocytochemistry

The histologic picture of the vessels after stent implantation was very similar in all groups. All stents were embedded in the vascular wall and covered with a layer of neointima of limited thickness. Immunocytochemical analysis showed that the luminal side was indeed covered by endothelium (Fig. 4). Smooth muscle cells in a collagen-rich matrix were the main constituent of the neointima. In the immediate vicinity of the wires, however, smooth muscle cells were replaced by another cell type showing the typical macrophage morphology and occasionally multinucleated giant cells (Fig. 5). Giant cells originate from fused macrophages and have a life span *in vitro* of approximately 1 week [37]. The presence of giant cells for as long as 12 weeks after implantation can, therefore, be explained by either continued local proliferation of macrophages or their continued migration from the flowing blood [38]. The frequently observed adhesion of leukocytes to the endothelial lining underscores the latter explanation (Fig. 6).

The lamina elastica interna was damaged to some extent in all cases, but tearing was an infrequent observation. The arterial media was compressed in all vessels but appeared normal otherwise. In only two cases the stent wire had lacerated the media, accompanied by a more extensive inflammatory reaction.

Scanning and transmission electron microscopy

All studied coronary segments containing stents were completely endothelialized at 12 weeks. The adhesion of leukocytes was frequently observed (Fig. 6). In addition, the endothelial lining in the proximal and distal ends of some of the stents exhibited

Table 5. Quantitative histologic measurements of thickness of the layers of the vessel wall

	Neointima thickness, *μm**		Media thickness, *μm**†	
Study group	Above wires	Between wires	Under wires	Between wires
Group A ($n=8$)	134(39–413)	120(47–352)	52(34–117)	106(57–149)
Group B ($n=7$)	101(73–192)	111(99–169)	77(55–94)	116(103–153)
Group C ($n=11$)	143(40–756)	120(68–536)	79(0–107)	127(94–229)
Group D ($n=8$)	113(44–167)	124(87–149)	70(53–105)	122(88–146)

*Values given are median, with range in parentheses.
†P values of all data points of groups B, C, or D versus group A are >0.10.

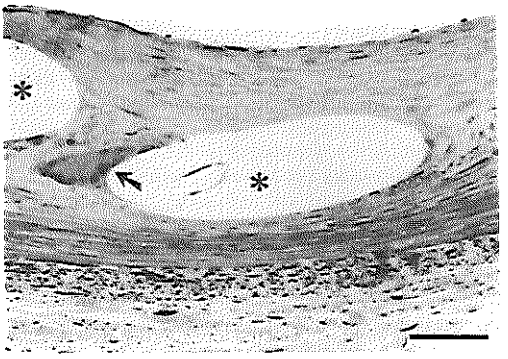

Fig. 5. Light microscopic picture of the neointima at 12 weeks showing a macrophage giant cell (*arrow*) adjacent to the stent filament (*asterisks*). *Bar* represents 50 μm.

areas with abundant surface folds (Fig. 7). This indicates enhanced endothelial pinocytosis or permeability. All side branches in the stented areas proved to be patent, and the wires crossing these side branches were in all instances covered by endothelium.

Discussion

Short-term patency of coated and uncoated self-expanding stents

The widespread clinical use of coronary endovascular stents remains limited by the need for intensive anticoagulation to prevent thrombosis [19,20,23]. Preliminary data from uncontrolled clinical studies in selected patients suggest that stents may improve the immediate angiographic results [39] and reduce both acute post-PTCA occlusion [21,22], as well as the incidence of late restenosis [19,24]. To be able to exploit fully these possible advantages of stenting, the problem of thrombogenicity has to be solved. Covering the stent filaments with a nonthrombogenic polymer layer may provide such a solution, by means of changing the surface chemistry or simply by smoothing the original surface (surface texture) [27,32].

The results of the present study show that attaching an ultrathin layer of hydrocarbons to the stent wires of stainless-steel self-expanding stents implanted in normal coronary arteries of pigs is at least as effective in preserving the short- and intermediate-term patency rates as deep anticoagulation with coumarin. Evidence for the efficacy of the polymer coating was most clearly demonstrated at autopsy by examination of the stented arteries of animals who died suddenly. In all animals, at least one of the stents was blocked by a typical arterial thrombus.

The smaller diameter (3.0 mm) stents did not show more frequent thrombotic occlusion than the larger stents (3.5 mm). Only five of the 13 stents with acute or subacute occlusion had a diameter of 3.0 mm (three of seven in group A, two of four in group B, and none of the two in group C). The uneven distribution of stent sizes, therefore, did not influence the differences in patency between the groups.

Pigs are very vulnerable to life-threatening arrhythmias after application of a significant coronary stenosis [40]. This offers the opportunity to detect transient thrombotic obstructions, which in other species may remain undetected.

In addition to the five pigs (nine occluded stents) that died suddenly within the first week, three animals had a total of four stents occluded between 1 and 4 weeks after implantation. Microscopic examination showed neointimal proliferation to be the cause in one case. In the other three stents the same macroscopic and microscopic picture of stent thrombosis was observed that was seen in the cases showing occlusion within the first week. This finding underscores the clinical observation of acute and subacute thrombosis [20]. Whether the continued treatment with aspirin or coumarin would have prevented these cases of subacute thrombosis is unknown, but it would not have influenced the main conclusions of this study.

We have shown earlier that stents are covered within 1 week by endothelium in pigs [25,41]. The present study indicates that this offers no guarantee against thrombotic occlusion, suggesting that some antithrombotic functions of this endothelial lining are not intact. Several additional indications for endothelial dysfunction within the stents were obtained. The dye exclusion test with Evans Blue showed signs of enhanced passive permeability of

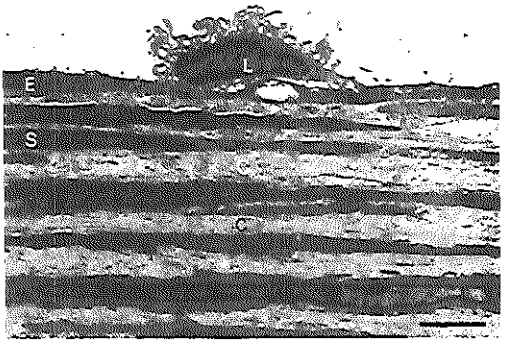

Fig. 6. Transmission electron micrograph of the neointima in a stented coronary segment at 12 weeks showing leukocyte adhesion (L) to the endothelial lining (E). *Bar* represents 2.5 μm. C—collagen; S—smooth muscle cell.

the endothelial lining (Fig. 2). In addition, the abundance of endothelial surface folds (Fig. 7) suggests that there is also enhanced active transport across the endothelial barrier. The frequently observed adhesion of white blood cells (Fig. 6) provides evidence that the endothelial cell membrane has undergone changes. Finally, we have recently shown that the metabolism of angiotensin in the neointima may be altered as late as 3 months after stenting [42].

Neointimal hyperplasia

Both quantitative angiography as well as histologic analysis showed a limited neointimal hyperplasia in the not acutely occluded, stented coronary arteries. Several factors may be responsible for this restricted tissue reaction. First, we implanted the devices in normal, nonatherosclerotic blood vessels with favorable flow characteristics [43]. Second, angiographic analysis showed that there was no mismatch between stent and vessel diameter, and third, only single stents were implanted in separate coronary arteries. Both considerable mismatch and the use of multiple stents were associated with a higher risk for developing restenosis after clinical implantation of the Wallstent [44].

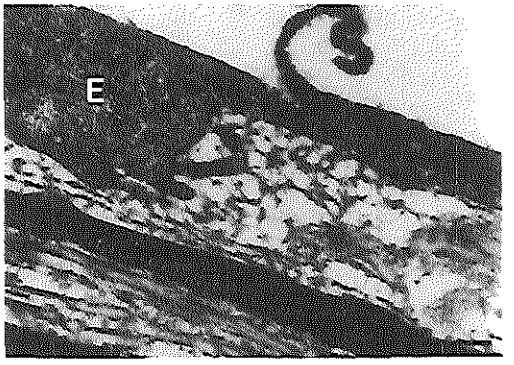

Fig. 7. Transmission electron micrograph of the endothelium within a stent at 12 weeks with surface folds protruding into the lumen. *Bar* represents 0.5 μm. E—endothelium.

Neither antithrombotic prophylaxis nor the polymer coating limited the extent of neointimal proliferation in our experiments. At least four explanations may be forwarded to interpret this finding. First, animals with significant thrombosis, which potentially may have harbored the trigger for enhanced proliferation, were lost from follow-up due to sudden death. This implies a selection bias in favor of cases with less thrombosis. Clinical experience with the Wallstent lends support to this reasoning: With advancing operator experience, the incidence of acute occlusion tends to decrease, but the occurrence of restenosis increases [24]. Second, the hypothesis that thrombotic stimuli are important for the genesis of neointimal proliferation may be false. Third, limited neointimal hyperplasia may be a phenomenon quite different from the extensive proliferative process leading to restenosis, initiated by distinct stimuli and inhibitors of growth. Finally, and quite likely, both "thrombosis-borne" and "vessel wall damage-born" factors act in concert during the development of restenosis, and the elimination of a single factor can not effectively inhibit this process [45]. Reversing this last argument, the most significant factor in the present study may have been that we avoided extensive vessel wall damage by matching the size of the stents to the dimensions of the recipient artery (Table 4). A large increase in lumen diameter by PTCA as determined by angiography has been shown to be related to restenosis in humans [46]. In addition, in an experimental model, histologically assessed damage, such as tearing of the internal elastic lamina and deep medial injury, was shown to be proportional to the severity of luminal narrowing [47,48]. Whatever the mechanism, the present finding that the Biogold polymer coating does not influence the process of neointimal hyperplasia is in agreement with a retrospective relative risk analysis of clinical implantations [44].

Conclusions

The data from the present study suggest that anticoagulant treatment with acenocoumarol or the BioGold polymer coating protects against early thrombotic occlusion of stainless steel self-expanding stents in normal coronary arteries of pigs. The extent of neointimal hyperplasia was limited in all groups and was not affected by preventive measures against stent thrombosis.

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Chapter 4

Histological features of a polymer endovascular prosthesis after transcatheter implantation in pigs

Histological Features of a Polymer Endovascular Prosthesis After Transcatheter Implantation in Porcine Arteries

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A novel approach for the treatment of acute complications and the prevention of restenosis after percutaneous transluminal angioplasty may be the placement of endovascular prostheses (stents). Stents constructed of metal have proven to be thrombogenic, and although they show a tendency to reduce restenosis, they do not prevent it. Pursuant to the search for stents with improved material and surface characteristics, we report in this paper the histological results obtained with a synthetic polymer (polyethylene terephthalate) stent after placement in porcine peripheral arteries. Eight stents were placed at a preselected site and resulted in a 87.5% angiographic patency rate at four weeks' follow-up examination. The neointima measured 114 ± 38 μm (mean ± SEM) on top of the fibers and 246 ± 44 μm between the fibers. Medial impression by the stent measured 27% ± 5%. The neointima consisted mainly of smooth muscle cells. A variable inflammatory reaction and foreign body response to the polymer was observed in all vessels. The present study shows the feasibility of the arterial implantation of polymer stents, in that they result in good intermediate-term arterial patency and limited neointimal hyperplasia. The observed inflammatory reaction, if prolonged, may limit the use of this polymer stent.

Percutaneous transluminal angioplasty (PTA) is an increasingly used treatment for obstructive atherosclerotic coronary and peripheral arterial disease. The initial success rate of PTA is better than 90%, but acute or subacute occlusion at the site of angioplasty occurs in 2% to 7% of coronary cases (1) and up to 15% in case of femoral and popliteal arteries (2). Aspirin can reduce acute occlusion, though other pharmacological therapy does not seem to be superior (1,3). Later, restenosis occurs in 20% to 50% of cases after successful PTA of coronary arteries and gradually develops within 3 to 6 months after the procedure (4). In peripheral arteries success, scored as patency, tends to be higher in aortoiliac arteries (85% patency at 5 years), whereas in femoral arteries patency gradually decreases to 58% at 5 years (5). Pharmacological treatment does not prevent restenosis (6,7). Vascular stents are currently studied as an adjunct to PTA to treat acute complications and to prevent restenosis (8). These devices become embedded in the vessel wall and are covered by endothelium within one week in coronary arteries in pigs, and at least within three months in human saphenous vein aortocoronary bypass grafts (9,10). Experimental and clinical studies with metal stents indicate, however, that these devices are thrombogenic and require a stringent anticoagulation regimen after implantation.

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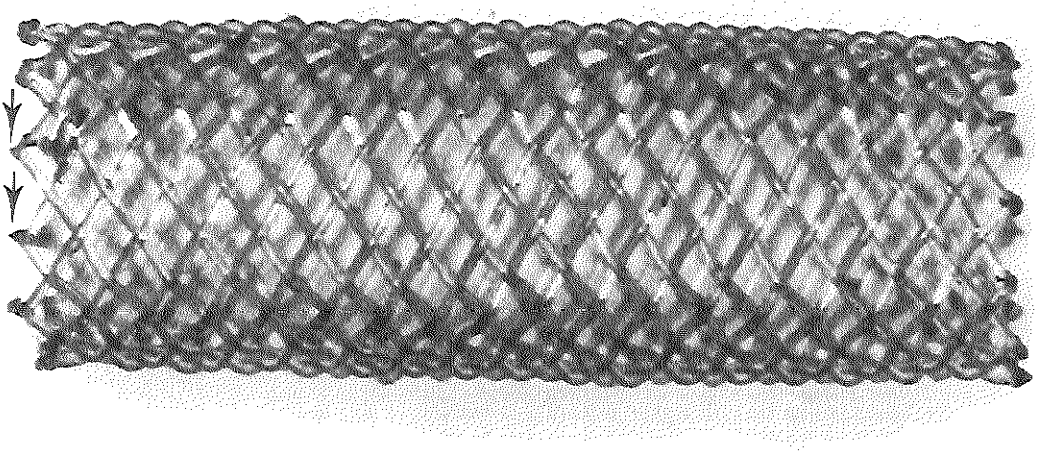


Figure 1. The fully expanded polyethylene terephthalate mesh stent has a diameter of 5.3 mm and a length of 15 mm. The wires (arrow), 176 μm thick, have been thermally fused to ensure that the device remains woven during and after the implantation procedure.

Although results of uncontrolled clinical studies indicate a lower incidence of restenosis as compared with conventional PTA, current stents do not eliminate this phenomenon (11–13). At present biocompatible polymers are advocated as a replacement for metal or to serve as a coating (14,15). Polymers such as polytetrafluoroethylene and polyethylene terephthalate are likely candidates because of their successful use as graft material for peripheral vascular surgery. In this paper we report the histological results obtained with a polyethylene terephthalate stent after transcatheter placement in porcine peripheral arteries.

Methods

Polymer stent. The stent is constructed as a cylindrical braided spring mesh, consisting of 24 polyethylene terephthalate (polyester, Akzo Fibres BV, Arnhem, the Netherlands) monofilaments, each 176 μm in diameter (Fig. 1). For implantation, this stent is mounted on a delivery system consisting of two coaxial catheters. After the system is positioned at the selected arterial site, withdrawal of the outer catheter will progressively release the stent, which anchors itself against the arterial wall. The mechanical properties of this stent have been described earlier (16).

Animal preparation. Experiments were performed in four Göttingen minipigs (24–28 kg). The protocol was approved by the Committee on Experimental Animals of Erasmus University. After anesthesia and under sterile conditions, an arteriotomy of the left carotid artery was performed, and a 9F introduction sheath was placed as described earlier (9). Then 5,000 IU heparin sodium was administered, and angiography of the arteries was performed. The angiograms were analyzed on-line using a quantitative cardiovascular angiography analysis system, and an arterial segment with a mean diameter of 3.5 mm was selected in seven femoral arteries and one carotid artery (17).

Stent implantation. The stent mounted on the delivery catheter was advanced through an 8F guiding catheter and over a 0.014-inch steerable guide wire to the site preselected for implantation. After an additional intraarterial administration of 2,500 IU heparin, the stents were placed.

After repeat angiography of the stented arteries, the arteriotomy was repaired and the skin closed in two layers. The animals were allowed to recover from anesthesia, but postprocedure antithrombotic drugs were not administered.

Harvesting of the test specimen. After repeat angiography at four weeks to assess patency, the recipient artery was cannulated and, after administration of a lethal dose of pentobarbital sodium, cross clamped proximal to the cannula. Through the cannula, 500 mL of saline, followed by 400 mL of buffered 4% paraformaldehyde, was infused. Thereafter the stented arterial segments were removed and placed in 4% paraformaldehyde or 4% paraformaldehyde plus 1% glutaraldehyde in phosphate buffer (pH 7.4) for at least 48 hours in preparation for light and electron microscopy, respectively, as described earlier (18). Specimens for light microscopy and immunocytochemistry were embedded in paraffin.

Morphometry. To measure the thickness of the various layers of the arterial wall, perpendicular resorcin-fuchsin-stained sections of the proximal, middle, and distal part of each stented segment were projected on a video screen, and the external and internal elastic lamina and the endothelial lining were traced using an integrated image analysis system (IBAS-2000, Kontron, Oberkochen, Germany) (19). The distance between the endothelial lining and the internal elastic lamina was taken as the thickness of the intima. The media was defined as the layer between the internal and external elastic lamina.

Immunocytochemistry. After rehydration, the sections were exposed to the horseradish peroxidase-labeled lectin of *Bandeiraea simplicifolia* (Isolectin B_4, Sigma Chemical Company, St Louis, MO) as an endothelial marker, and to monoclonal mouse anti-smooth muscle cell (SMC) specific α-actin (Sigma Chemical Company, St Louis, MO), monoclonal mouse antiporcine vimentin, and mouse antiporcine desmin (Dakopatts, Glostrup, Denmark) as an indicator of smooth muscle cell phenotype. As a second antibody, horseradish peroxidase-labeled rabbit antimouse antibodies were used (Dakopatts, Glostrup, Denmark). As a detecting agent, 700 μg/mL diaminobenzidine (Sigma Chemical Company) in phosphate-buffered saline was used.

Light microscopy. Qualitative light microscopical examination (BH2, Olympus, Tokyo, Japan) was performed in the proximal, middle, and distal part of the stented segments, as well as adjacent segments, using hematoxylin-azophloxin as a routine stain and resorcin-fuchsin as an elastin stain. Adjacent segments served as controls for nonstent (delivery system) inflicted damage.

Scanning electron microscopy. Scanning electron microscopy was performed on two properly stented arteries using methods described earlier (9). In short, the vessels were postfixed with 0.1 M cacodylate buffer containing 1% OsO_4 and 50 mM ferricyanide ($K_3[Fe(CN)_6]$) for at least 6 hours. After rinsing the specimens in 0.1 M cacodylate buffer, they were dehydrated in ethanol, critical-point dried in liquid CO_2, and sputter coated with gold. In addition, one stent that was not implanted was only sputter coated. After mounting, the specimens were examined using a JSM 25 (Jeol Ltd., Tokyo, Japan).

Statistical analysis. All quantitative data are expressed as mean ± standard error of the mean (SEM).

Results

In vivo implantation. Placement for all eight stents seemed easy at the preselected arterial sites. After four weeks one stented femoral artery proved to be angiographically occluded. An unexpected finding at autopsy was that in one animal both stents were found patent upstream of the intended site of implantation at the aortic bifurcation, extending into, but not occluding, the origin of both iliac arteries.

Quantitative histological measurements. The neointima, as measured on top of the polymer fibers, had a thickness of 114 ± 38 μm and, between the fibers, 246 ± 44 μm ($n = 5$). The media under the fibers had a thickness of 132 ± 41 μm, and between the fibers 173 ± 41 μm ($n = 5$). On average the media under the fibers was compressed by 27% ± 5% as compared with the media in between the fibers, as illustrated in Figure 2.

Immunocytochemistry. Labeling with *Bandeiraea simplicifolia* confirmed the presence of endothelium at the luminal surface. Anti-SMC specific α-actin antibodies stained most cells in both the neointima and the media. Staining for vimentin and desmin to identify SMC contractile phenotype was, contrary to our expectation, negative for both antibodies, with the exception of a few cells in the media. Medial smooth muscle cells in the segments directly adjacent to the stents, however, did stain positively for desmin.

Morphology. Macroscopically, the patent stents were covered with a thin, almost translucent layer of tissue (Fig. 3). Microscopical examination of the vessels showed that underneath the stent fibers, the media was compressed and acellular to various extent. Other ab-

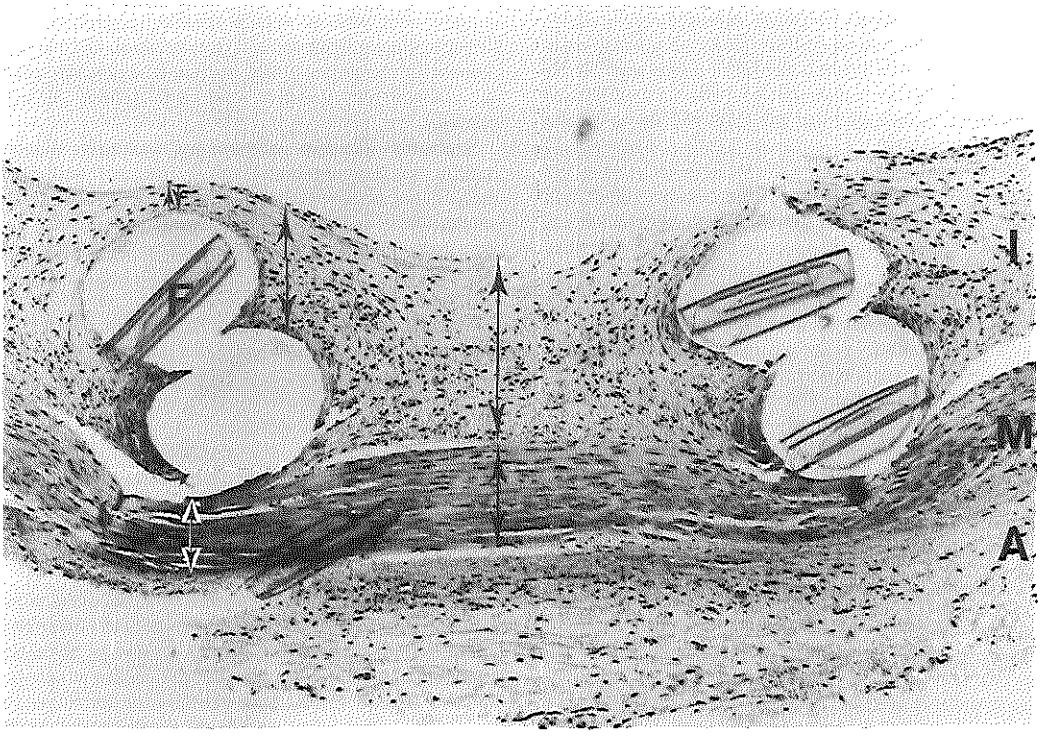


Figure 2. Detail of a femoral artery 4 weeks after stenting. For morphometric analysis, the thickness (between arrows) of the neointima (I) and media (M) of the stented segments was measured on top of and below the fibers (F) as well as between the fibers. A: adventitia. (Hematoxylin-azophloxin stain, ×93.)

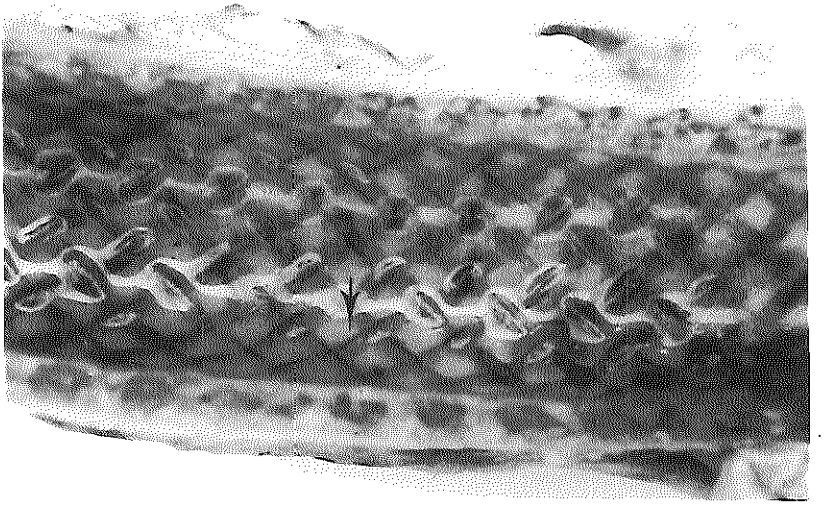


Figure 3. Macroscopy of a femoral artery, 4 weeks after stenting, showing a thin, translucent layer of tissue covering the polymer fibers (arrow).

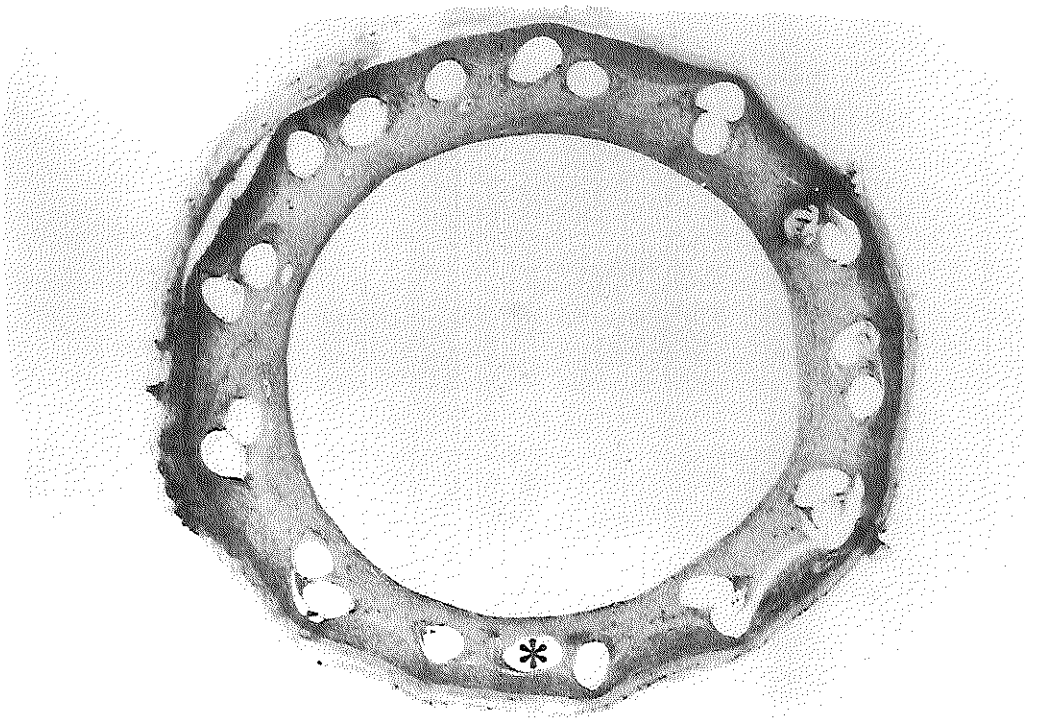


Figure 4. Microscopic overview of a femoral artery 4 weeks after stenting. The stent fibers (* = stent fiber void) are completely covered by a neointimal thickening, consisting of smooth muscle cells within a collagenous matrix and covered by endothelium. (Hematoxylin-azophloxin stain, ×24.)

normalities of the media, such as inflammatory reactions, were less frequently observed. The appearance of the intima, however, varied considerably, as described in the following paragraphs.

Neointimal thickening. Some variability existed in the amount of intimal thickening, which consisted of SMC within a collagenous matrix (Fig. 4). The proximal and distal adjacent control segments did not reveal any intimal thickening. Around the stentwire voids, some fibrin remnants were observed, as well as some hemosiderin-filled macrophages.

Inflammation. In general, an inflammatory infiltrate within the neointima partly overlay the polymer fibers and was covered by a fibrous cap, consisting of SMC in a collagenous matrix that was sometimes heavily vascularized. In some vessels, or parts of them, the inflammatory response was absent or consisted of a limited number of leukocytes, whereas in others a very extensive reaction was found (Fig. 5), consisting predominantly of eosinophilic granulocytes and macrophages, organized into nodules (granulomatous inflammation).

Foreign body response. Multinucleated giant cells were present next to the polymer fibers in almost all vessels, showing that a foreign body response to the stent was elicited. In addition, these cells were found surrounding polymer inclusions in the neointima (Fig. 6).

Thrombus organization. In the case of the almost occluded vessel, we found not only a neointima, but also a new layer of circularly oriented smooth muscle cells bordered by an elastic membrane, resembling a neomedia (Fig. 7), as well as numerous hemosiderin-filled macrophages and extensive neovascularization.

Scanning electron microscopy. Examination of the stented arteries showed a largely intact endothelial layer at four weeks (Fig. 8). The endothelial cells exhibited a cobblestone morphology. Some leukocytes and platelets were adherent to denuded patches. Scanning electron microscopy of a stent that was not implanted revealed a fiber surface that was principally smooth. It was noticed, however, that the wires were

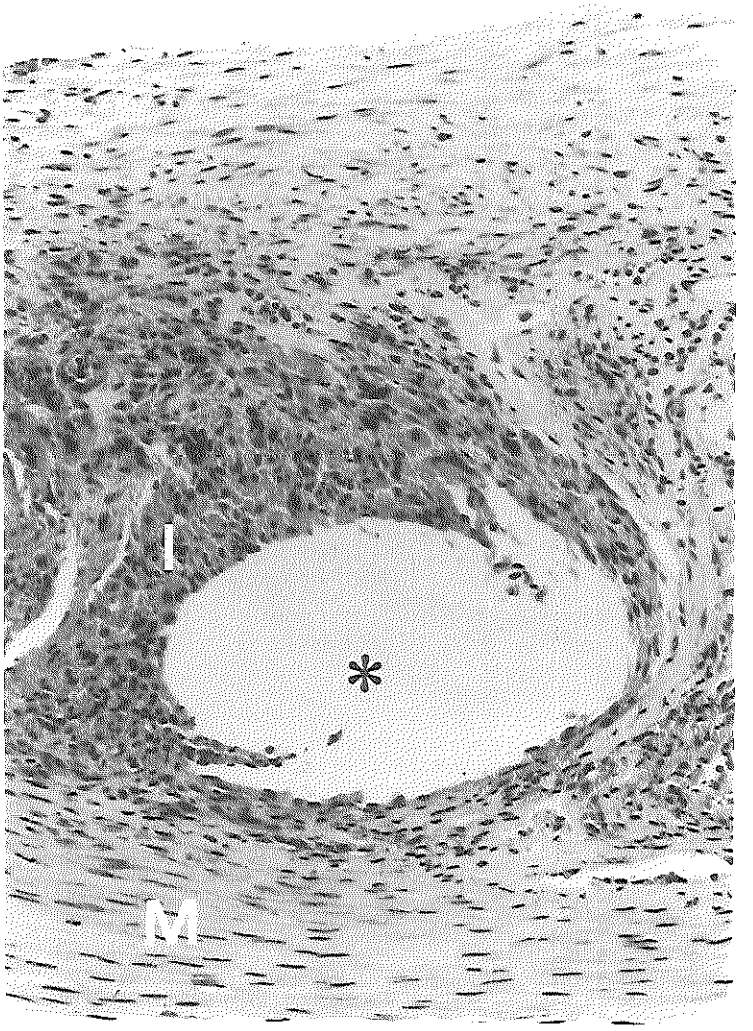


Figure 5. Detail of a stented artery, 4 weeks after the implantation procedure, showing inflammatory infiltrates (I) in the neointima overlaying the polymer fibers(*). M: media. (Hematoxylin-azophloxin stain, ×186.)

damaged at several places resulting in deep crazes (Fig. 9A), large protuberances, and loose threads (Fig. 9B).

Discussion

Choice of polymer for construction of the stent. Polyethylene terephthalate (PETP, also known as Dacron®, Terlenka®, Terylene® and Diolen®), is the most frequently used material for large-caliber peripheral vascular grafts and has primary patency rates as high as 74% after 10 years (20). The mechanical properties of drawn PETP fibers can be modified considerably, depending on processing conditions, therefore enabling us to choose the force with which this type of endovascular prosthesis will expand and anchor itself against the vessel wall.

Several mechanical and chemical properties dictate the choice of a material for use as a endovascular prosthesis. For instance, different clinical indications may require different radial forces exerted by these prostheses. In order to prevent elastic arterial recoil, a more rigid stent is needed than when the stent is intended only to tack back dissection flaps to improve the results

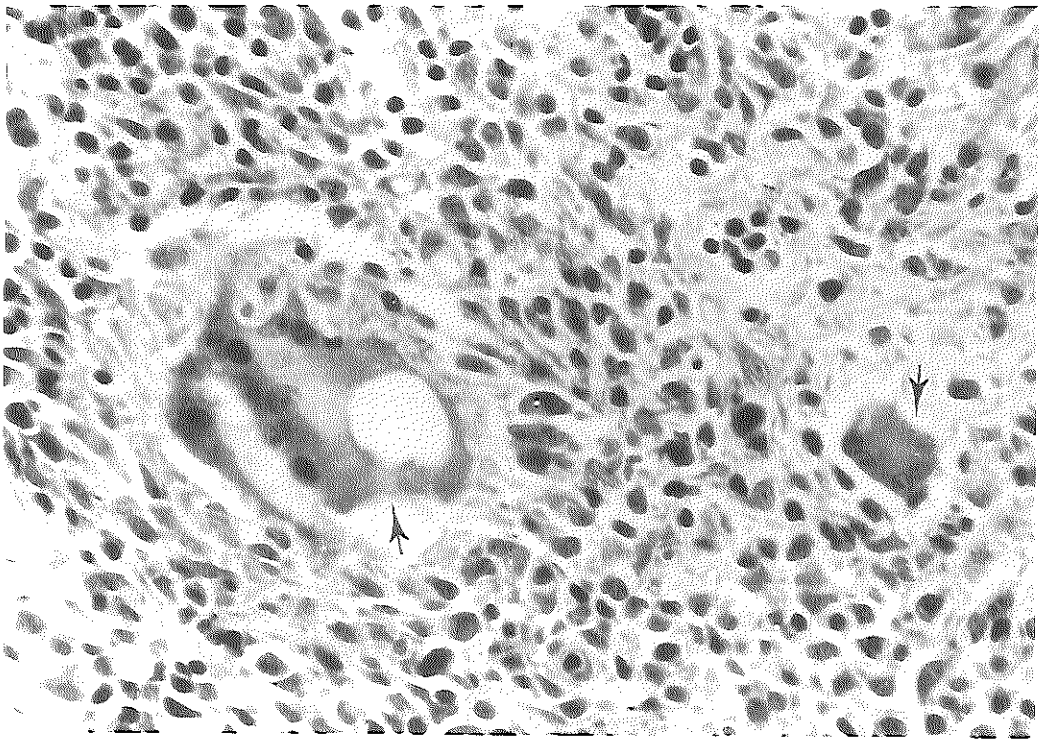


Figure 6. Detail of a stented artery, 4 weeks after the implantation procedure, showing multinucleated giant cells (arrows) surrounding polymer fibers. (Hematoxylin-azophloxin stain, ×490.)

of percutaneous transluminal angioplasty. Intralesion morphology (calcium vs. lipid gruel) may be an additional determinant. Furthermore, the design of the stent and its mode of delivery also demands certain material prerequisites. The radial force of the prosthesis should, however, be limited to prevent pressure necrosis and late restenosis, which has been found to be related to the implantation of oversized stents (21,22).

The material has to be biocompatible, nonthrombogenic, and capable of being endothelialized. In addition, the surface has to be smooth in order to avoid the retention of surface air bubbles, which will make the surface of even a nonthrombogenic material highly thrombogenic. A rough surface will also promote macrophage adhesion and induce a foreign body reaction, which can delay wound healing and endothelialization because of excreted products (23).

Morphometry. Quantitative microscopic examination showed a limited though variable amount of neointimal proliferation in the polymer stents at four weeks. Although a certain amount of neointimal thickening is attributable to the normal process of wound healing after mechanical damage inflicted by the stent, and is similar for all stents, we did observe some significant differences between the several stents that are currently available and the stent studied in the present study (e.g., with respect to neointimal thickening). The thickness of the neointima on top of the polyester stent fibers was similar to that observed after implantation of a balloon-expandable tantalum stent, but it is considerably better than that produced by a balloon-expandable stainless steel stent, implanted under similar conditions and in the same species (9,24) but in the coronary circulation.

Immunocytochemistry. Positive staining with *Bandeiraea simplicifolia* revealed that the neointima was lined by endothelium. How well the endothelium functions as a physical barrier, its effectiveness in producing and excreting vasoactive substances such as EDRF, and their propensity in reacting to external stimuli is not known but may be lacking in several aspects

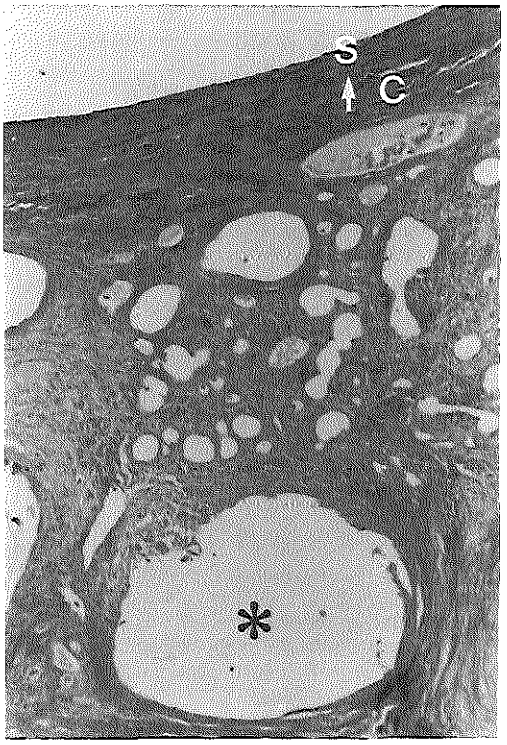


Figure 7. Detail of the almost-occluded artery, showing a layer of smooth muscle cells (S) at the lumen, bordered by an elastic membrane (arrow) resembling a neomedia. Underneath is a thick layer of collagen (C) having a wavy appearance; * = stent fiber void. (Resorcin-fuchsin stain, ×129.)

(25,26). Smooth muscle cells represented the main cellular component of the neointima. Remarkably, we observed that the medial smooth muscle cells within the stented segment did not stain for desmin, a cytoskeletal protein usually present in contractile SMC, whereas the directly adjacent nonstented segments did stain positively. Vimentin stained negative in both cases. Whether or not the loss of desmin expression indicates a loss or change in contractile function is unclear.

Light and electron microscopy. Considerable differences in the histological appearance of the neointima were observed in the present study when compared with the results obtained with metallic stents in the peripheral or coronary circulation. Contrary to results with metal stents, an extensive inflammatory and foreign body response surrounded the embedded polymer fibers. In some arteries we observed inflammatory cells invading the polymer fibers or surrounding inclusions of polymer separate from the fibers. The latter observations, confirmed by scanning electron microscopy, underscore the requirement for "gentle" production techniques of polymer stents.

Although one report on the intravascular use of Dacron mounted on an intravascular prosthesis was very optimistic in that a limited amount of neointimal hyperplasia was found without signs of inflammation or foreign body reactions (27), our observation concerning the inflammatory infiltrates are in agreement with that reported by Maturri et al. (28). Perhaps the vascular response is dependent on the chemistry of the polymer (e.g., use of metal salts as catalysts for esterification, such as titanium or antimony salts) as well as the addition of plasticizing agents that can enhance the handling properties of the polymer. These agents remain present in the polymer as traces and, theoretically, can therefore diffuse from the fibers into the tissue and there elicit a toxic response.

Sterilizing methods have also been shown to be an important determinant in the healing response (29,30). Immediately before implantation, we sterilized the stents in a Cidex solution containing 2% glutaraldehyde, or in chlorhexidine in ethanol, for 15 minutes, followed by a rinse in saline. Theoretically, PETP may absorb up to 10 to 100 ppm of glutaraldehyde and up

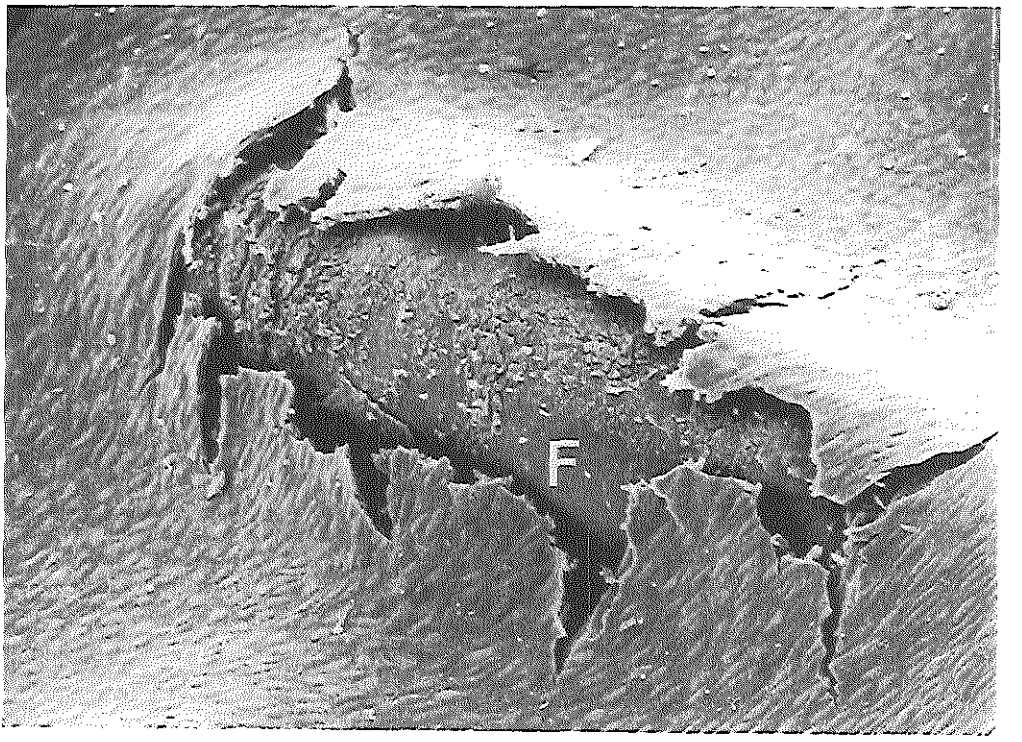
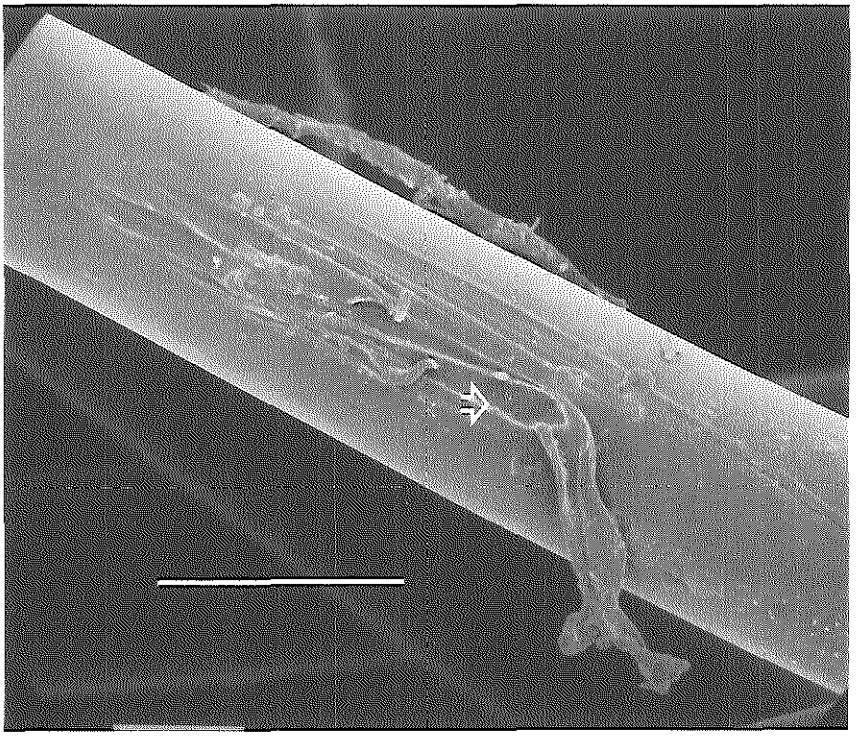


Figure 8. Scanning electron micrograph of a stented artery, 4 weeks after the procedure, showing an endothelial lining covering the stent. The thin intimal lining has cracked during preparation for microscopy, laying bare the polymer fiber (F) underneath. Some leukocytes (arrow) can be seen adhering to the endothelium (×150).

to 0.01% of ethanol during the immersion period. The inflammatory tissue reaction observed in our study is likely to have been aggravated by the local irritant effect of glutaraldehyde and/or ethanol-derived acetaldehyde after diffusion in the vessel wall. Better results can be expected when sterilization is performed with the use of gamma irradiation, as was shown by Guidon et al. (30). Our experiments may therefore be regarded as being performed in a worst-case environment. In the one subtotally occluded stent the observations are indicative of thrombotic complications, as illustrated by the "neomedia" phenomenon, which is typical for the organization of thrombus deposition (31) and is confirmed by the presence of hemosiderin and extensive neovascularization, which also indicates a thrombotic event during or after stenting.

Conclusions. Although we have to take into account that these studies were performed in arteries of young healthy animals, whereas the intended use is for atherosclerotic arteries of usually older patients, the present study shows the feasibility of the arterial implantation of polymer stents with good intermediate-term results in patency and neointimal proliferative response. Our results differ from preliminary reports on porcine coronary implants, which were also technically feasible but showed complete occlusion at four weeks for all cases (32). The difference in experimental outcome may be explained by any of the aforementioned factors, such as polymer chemistry and sterilization. A differential response to injury between peripheral arteries and coronary arteries may theoretically be responsible but is not very likely. The small caliber of coronary arteries compared with femoral and carotid arteries and the differences in flow patterns are a more likely cause for the tendency for thrombotic occlusion.



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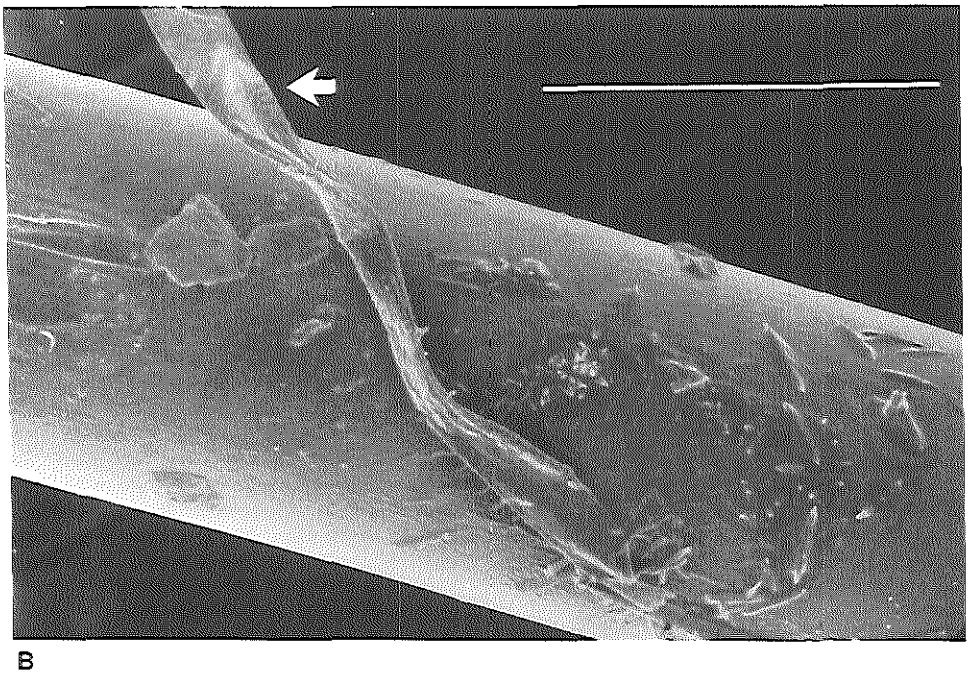

B

Figure 9. Scanning electron micrograph of the not-previously-implanted stent, showing **A** deep cracks (arrow), large protuberances, and **B** loose threads (arrow) barely attached to the stent. (Bar = 176 μm.)

The abundant inflammatory reaction, if prolonged and associated with local vascular dysfunction, could limit the use of this polymer stent. Further research into the material used for this type of prosthesis and its chemical processing should yield better results in the future.

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Chapter 5

Stenting or balloon angioplasty of stenosed autologous saphenous vein grafts in pigs*.

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ABSTRACT

Stents may be useful as an adjunct to balloon angioplasty of narrowed saphenous vein grafts. We compared the angiographic and histological outcome of balloon angioplasty and a balloon-expandable stent in a model of early vein graft stenosis in pigs. In 31 animals a piece of overdistended autologous saphenous vein was grafted end-to-end to replace an excised segment of both carotid arteries. Angiography after 4 weeks revealed that 45% of the grafts were occluded. In the patent but narrowed grafts an intervention was performed with a single stent (n = 12 grafts), multiple stents (n = 6), or balloon angioplasty (n = 6), while grafts with mild stenoses were left untreated (n = 8). Four weeks after this intervention, angiography showed that grafts with single stents, balloon angioplasty or untreated grafts had patency rates of 92%, 83% and 83%, respectively. Grafts receiving multiple stents, however, showed only a 17% patency rate ($p<0.05$). Quantitative analysis of the angiograms showed that balloon dilatation or placement of a single stent acutely improved the minimal diameter by 0.6 ± 0.2 and 0.8 ± 0.3 mm, respectively. This initial gain was lost, however, during the follow-up period. Multiple stents showed a similar gain (0.5 ± 0.2 mm) but more loss during the follow-up period (2.4 ± 0.2 mm). Histology showed a limited neointimal build-up both in treated and untreated grafts. Some stents were only partly embedded in the graft. Occluded grafts showed signs of organized thrombus in all treatment groups.

In conclusion: single stents and balloon angioplasty show good patency in early saphenous vein graft narrowing, but multiple stents show a high occlusion rate.

INTRODUCTION

Coronary artery bypass graft (CABG) operations, using autologous saphenous veins are a widely employed surgical therapy for obstructive atherosclerotic coronary artery disease. Up to 12% of grafts, however, occlude within one month after the operation with an additional 10% occlusion rate during the first year. A subsequent yearly occlusion rate of 2% has been reported, resulting in a 65% patency rate at 5 years [1,2]. Repeat bypass surgery is feasible in most cases, but is associated with an increased morbidity and mortality, while long term patency is inferior to the first procedure [3,4,5]. Moreover, a significant number of patients are, for several reasons, poor candidates for repeat surgery [6]. Percutaneous transluminal coronary angioplasty (PTCA) may be an alternative to surgery in these patients. The initial success rate of this procedure is high (90%), but the restenosis rate after PTCA in bypass grafts is considerable, and only 41% of patients are alive and event-free after two years [7]. However, not all patients are candidates for balloon angioplasty [6].
The implantation of endovascular prostheses, also called stents, seems a promising new technique for treatment and prevention of restenosis in these patients [8,9]. The angiographic and histological results after stent implantation in bypass grafts have only been reported to a limited extent [9,10,11]. We studied, therefore, the placement of stents in an animal model for autologous saphenous vein grafting [12], and compared the results with those of balloon angioplasty alone, or no intervention.

METHODS

Vein graft model Experiments were performed in 31 Yorkshire pigs (17-30 kg; HVC, Hedel, The Netherlands). The protocol was approved by the *Committee on experimental animals* of the Erasmus University. After an overnight fast the animals were sedated with 20 $mg.kg^{-1}$ ketamine hydrochloride (ketalin, Apharmo BV, Arnhem, The Netherlands). Following endotracheal intubation, the pigs were connected to a ventilator which administered a mixture of oxygen and nitrous oxide (1:2, v/v). Anesthesia was maintained with 1-4 vol % enflurane (ethrane, Abbot BV, Maalderij, The Netherlands). Antibiotic prophylaxis was administered by an intramuscular injection of 1000 mg of a mixture of procaine penicillin-G and benzathine penicillin-G (Duplocillin, Gist-Brocades NV, Delft, The Netherlands). The implantation of two venous grafts was performed according to the methods described by Angelini et al. [12] (Fig. 1). Briefly, skin incisions were made parallel to, and approximately 3 cm lateral to the trachea on both sides. Both common carotid arteries were prepared free over a length of approximately four centimeters, and subsequently covered with a gauze soaked in a 1% papaverine solution (paparini sulfas, Centrafarm BV, Etten-leur, The Netherlands), to prevent vasospasm. The vena saphena minor of the left leg was prepared free over a distance of approximately 5 cm, and the larger side branches secured. After removal, the vein was overdistended using a syringe, containing a solution of 1% papaverine and 10 U/ml heparin (thromboliquine, Organon Teknika BV, Boxtel, The Netherlands). Thereafter two pieces of vein, approximately 15 mm in length, were excised and stored in the same solution. After cross clamping of one carotid artery, a 15 mm piece of the vessel was removed, and replaced by a piece of saphenous vein by two end to end anastomoses using 6.0 prolene (ethicon, Norderstedt, Germany). After intravenous administration of 2.500 U heparin, the clamps were released. The contralateral carotid artery was grafted in an identical way. The anastomotic sites were marked with stainless steel hemoclips. The neck and leg wounds were closed in layers using catgut (Chronic Gut, American Cyanamid Co, USA) and the skin covered with antiseptic spray (Nobecutane, Bofors, Nobel-Pharma, Sweden). Thereafter, the animals were allowed to recover from anesthesia. Two animals died during the first days after surgery.

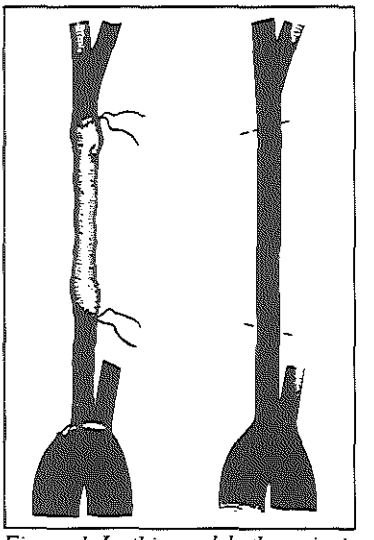

Figure 1 In this model, the vein is interposed in the carotid artery (courtesy of Prof. GD Angelini, Bristol, UK).

Angiography Four weeks after surgery 29 animals were anesthetized as described above. Under sterile conditions an arteriotomy of a femoral artery was performed and a 9F introduction sheath was inserted. Then 5.000 IU heparin sodium was administered and an 8F guiding catheter was advanced to the aortic arch. After measurement of arterial blood pressure and heart rate, and withdrawal of an arterial blood sample for the measurement of blood gases and acid-base balance (settings of the ventilator corrected, if necessary), angiography of the grafted carotid arteries was performed using Iopamidol (Iopamiro^{R} 370, Dagra, Diemen, The Netherlands) as a contrast agent. Only animals with at least one patent graft were used for further study. One animal was excluded from the study because of infection. In this animal, the acute effect of stent placement was studied by scanning electron microscopy.

Balloon-expandable stent The balloon-expandable stent used in this study (Wiktor™, Medtronic Inc., Minneapolis, USA) is constructed of a single tantalum wire (0.127 mm in diameter) which is formed into a sinusoidal wave and wrapped into a helical coil structure. This prosthesis is crimped onto the deflated polyethylene balloon of a standard angioplasty catheter. The features of this prosthesis design are such that by inflating the balloon the diameter of the stent increases without alteration of its length [13]. The maximal diameter of the balloon after inflation determines the ultimate size of the prosthesis after implantation. One inflation at 8 atmospheres is sufficient to open the stent and allows the safe withdrawal of the deflated balloon. The balloon-diameters of the mounted angioplasty catheters used in this study were 4.0 mm, and the lengths of the prostheses 14-16 mm.

Intervention From the angiograms, the patent grafts were divided into four groups by visual assessment (Table 1). A first group of 8 grafts (12 anastomotic sites) were considered to have mild stenoses and an intervention was not performed in these grafts to allow the study of progression of vein graft hyperplasia. A second group of 6 grafts were dilated by a 4.0 mm angioplasty balloon up to 8 atmospheres for 40 seconds. A third group of 12 grafts received a single 4.0 mm stent covering the most narrow arteriovenous anastomosis. A last group of 6 grafts received 2 or 3 stents covering both anastomotic sites and the body of the graft. The implantation procedure of the 4.0 mm balloon-expandable stents was similar to the procedures as described earlier for the coronary circulation [13]. After stent implantation and balloon dilation repeat angiography was performed, the guiding catheter and the introducer sheath were removed, the arteriotomy repaired and the skin closed in two layers. The animals were allowed to recover from anesthesia. Antithrombotic drugs were not administered at any time during the follow-up period.

Intervention group	Animals (n)	Grafts (n)
No intervention	7	8
Balloon angioplasty	6	6
Single stent	12	12
Multiple stent	6	6

Table 1

Follow-up angiography and harvesting of the specimen Follow-up angiography was performed after 31 days, using the same projection and identical geometry of the X-ray gantry as during implantation. Thereafter the carotid arteries were dissected free. After cross-clamping and puncturing of the proximal part of the arteries, 500 ml of saline followed by 400 ml of buffered 4% paraformaldehyde was infused under a pressure of approximately 120 mmHg. The specimen were placed in 4% paraformaldehyde for light microscopy, or 4% paraformaldehyde plus 1% glutaraldehyde in phosphate buffer (pH 7.3) for at least 48 hours in preparation for electron microscopy.

Angiographic analysis Angiograms (performed immediately before and after intervention and again after 31 days) were analyzed off-line using the quantitative coronary angiography analysis system (CAAS) [14]. The minimal luminal diameter at the site of the intervention was measured.

Microscopical analysis After fixation, the graft-containing arterial segments were divided lengthwise into two equal parts using a pair of fine scissors. One part washed in 0.1 M

cacodylate buffer (pH 7.3), and postfixed in 1% OsO_4 in 0.1 M cacodylate buffer for at least 6 hours. After washing overnight in 0.1 M cacodylate buffer, the specimen were placed in 1% tannic acid for 60 min, 1% sodium sulfate for 10 min and again washed in 0.1 M cacodylate buffer. After dehydration in graded ethanol series and an overnight incubation in uranyl acetate/ethanol (7 parts ethanol 100%, 3 parts uranyl acetate saturated in aqua dest), the specimen were critical point dried with liquid CO_2 mounted on a specimen table and sputtercoated with gold before examination in a scanning electron microscope (ISI-DS-130, Akashi Beam Technology, Tokyo, Japan).
For light microscopy and immunocytochemistry, ± 3-4 mm wide transverse sections of the second part of the vessels were made with a pair of fine scissors and a sharp razor blade. With tweezers and with the aid of a dissection microscope, the metal stent wires were carefully removed and the tissue was dehydrated and embedded in paraffin. After sectioning and staining, microscopy was performed using a light microscope (BH2, Olympus, Tokyo, Japan). Hematoxylin-azophloxin was used as a routine stain, resorcin-fuchsin as an elastin stain and Goldner as a trichrome stain.

Immunocytochemistry After rehydration, the sections were exposed to monoclonal mouse anti smooth muscle cell (SMC) specific α-actin (Sigma Chemical Company, St Louis, M.O., U.S.A.), mouse monoclonal anti porcine vimentin and mouse anti porcine desmin (Dakopatts, Glostrup, Denmark) as a marker for smooth muscle cells. As a second antibody, Horse Radish Peroxidase-labelled rabbit anti mouse antibodies were used (Dakopatts, Glostrup, Denmark). As a detecting reagent 700 μg/ml Diamino Benzidine (Sigma Chemical Company) in phosphate buffered saline was used.

Statistical analysis All data are expressed as mean ± SEM, unless otherwise stated. Differences in patency between the intervention groups were analyzed by X^2-test. The significance of the changes in the angiographic data were evaluated by Duncan's new multiple range test once an analysis of variance (ANOVA) revealed the samples represented different populations (random block design). A P value < 0.05 was considered significant.

RESULTS

Vein graft model Repeat angiography 31 days after surgery showed that 32 (20 animals) out of a total of 58 grafts were angiographically patent (45% occlusion rate). All occluded grafts were blocked over their entire length, and were excluded from further study.

Hemodynamics during intervention Immediately before intervention in the grafts, heart rate was 105 ± 5 beats.min^{-1}, and systolic and diastolic arterial blood pressure measured 129 ± 5 and 93 ± 5 mmHg, respectively.

The acute effect of stenting This was studied by scanning electron microscopy in one animal at 4 weeks after surgery. The luminal appearance of the vein graft had a waveform geometry after placement of the stent. Due to the open sinusoidal configuration, parts of the vessel wall, not covered by the wires, were bulging out into the lumen (Fig. 2A). At a higher magnification an undulating surface was seen which had an undamaged appearance and was lined by endothelium. Damage to the vessel wall was only observed at the site where previously the stent wires had been present (Fig. 2B).

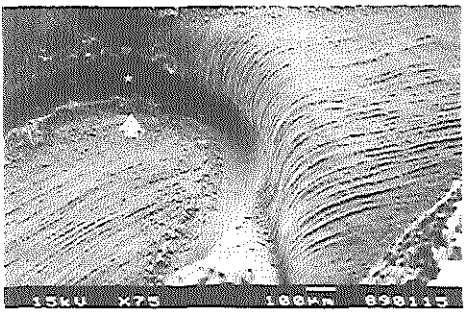

Figure 2A SEM 2 hours after stenting. Thrombus remnants (arrow) are in close apposition with the stent trenches (asterisk).

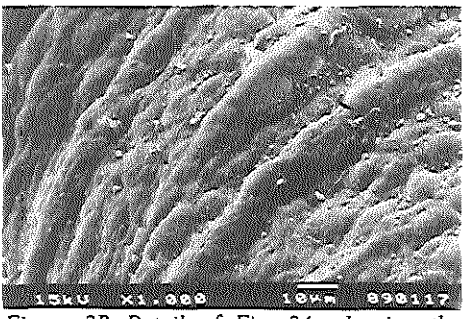

Figure 2B Detail of Fig. 2A, showing the apparently undamaged endothelium just above the stentwire trenches.

Angiographic patency four weeks after intervention Qualitative assessment of the angiograms of the four groups revealed that the patency rates after 4 weeks of follow-up of the groups receiving no intervention, receiving balloon dilation, or a single stent were 83%, 83% and 92%, respectively. In the group receiving multiple stents, however, only 17% proved to be patent ($p<0.05$ vs all other groups).

Quantitative angiographic analysis In all grafts the minimal luminal diameter (MLD) was localized at an anastomotic site. The data (Figure 3A) show that the mean MLD's of the intervention groups were smaller than the no-intervention group, but not significantly different.

Balloon dilatation, as well as the placement of single or multiple stents resulted in a similar immediate improvement of the mean angiographic MLD (0.6 ± 0.3, 0.8 ± 0.3 and 0.5 ± 0.2 mm, respectively, Figure 3B). After four weeks of follow-up, no change was observed in the mean MLD of the group without intervention (0.1 ± 0.8). All intervention groups showed a significant deterioration of the post intervention MLD ($p<0.05$), resulting in the loss of the initial gain. Although this loss during follow-up tended to be smaller for the single stent group (0.5 ± 0.8) compared to the balloon group (0.6 ± 0.6), the net difference between the groups did not reach levels of statistical significance.

The loss in MLD of the multiple stent-group (2.4 ± 0.2 mm), however, was significantly larger when compared to all other groups (Figure 3B, $p<0.05$), because of the high occlusion

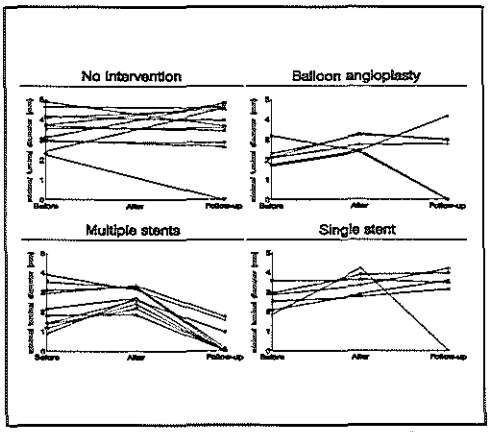

Figure 3A. The individual data showing the mean minimal luminal diameter (mm).

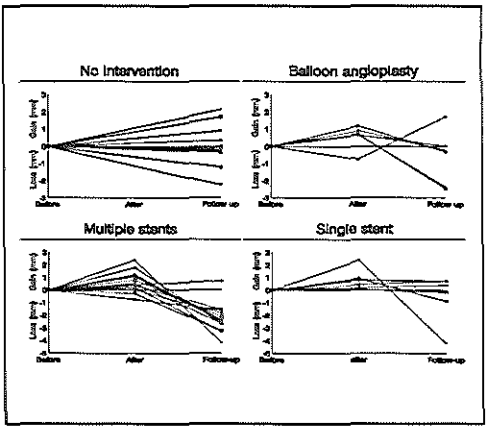

Figure 3B. The individual data showing the loss and gain in minimal luminal diameter (mm).

rate of 83%. When considering only those grafts that remained patent throughout the study, we found that, both for balloon angioplasty and single stenting, there was no decrease in mean MLD throughout the follow-up period. Only multiple stenting was associated with a net decrease in mean MLD (by 1.9 ± 0.3 mm).

Histology of the vein grafts The gross morphology of the grafts was variable. Both tight stenoses at the anastomoses, and aneurysmal dilatation of the body of the grafts were frequently observed. The adjacent carotid artery was usually normal. In all treated as well as untreated grafts, we observed both medial and neointimal thickening, which consisted of smooth muscle cells (confirmed with a positive staining for SMC-specific α-actin with immunocytochemistry) in a collagenous matrix (Figure 4). Most graft segments contained hemosiderin filled macrophages and inflammatory infiltrates. At the anastomoses, we observed inflammatory reactions, surrounding the prolene suture and the hemoclip. Macrophages were found containing crystalline inclusions, indicating that the inflammatory response was indeed directed to the prolene suture. In the single stent treatment group, we observed that four weeks after stent implantation thrombus remnants, surrounding the metal wires, were conspicuously present (Figure 5). The metal wires were not always embedded in the vessel wall (Figure 6).

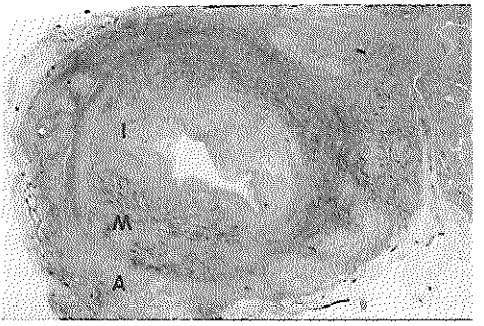

Figure 4 An overview of a narrowed vein graft. The completely endothelialized graft shows both medial (M) and intimal (I) hyperplasia.

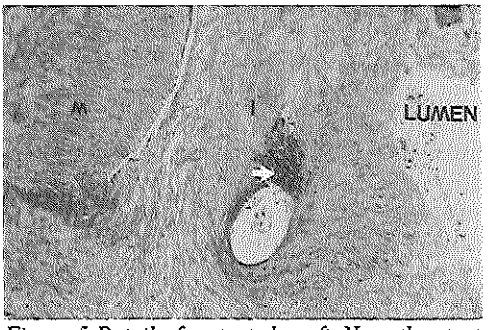

Figure 5 Detail of a stented graft. Near the stent voids, thrombus remnants are still visible (arrow).

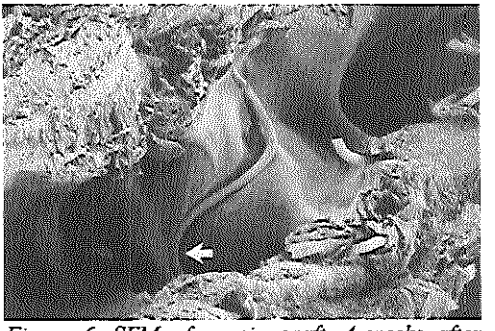

Figure 6. SEM of a vein graft, 4 weeks after implantation of the stent. Not all wires have become embedded in the vessel wall (arrow).

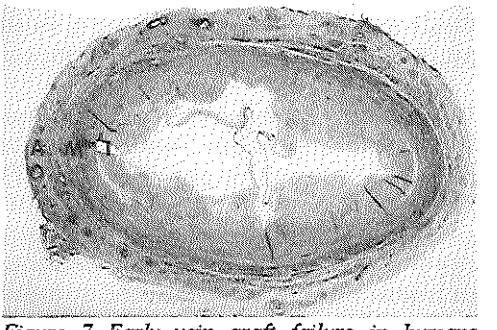

Figure 7 Early vein graft failure in humans resembles that in pigs. I intima, M media, A adventitia. Goldner trichrome magnification 10x.

Also, inflammatory infiltrates surrounding the stent wires were observed in the neointima, as well as occasional multinucleate giant cells. The adjacent carotid arterial side of the anastomosis, was less affected.

DISCUSSION

Porcine model of autologous saphenous vein grafting The autologous saphenous vein is still the most common conduit for aortocoronary bypass grafts in humans. The vein graft model used in this study (overdistended V. Saphena Minor, interposed in the A. Carotis Communis by two end-to-end anastomosis) [12] shows extensive intimal hyperplasia, that is histologically similar to early graft failure in humans (Figure 6). In this model we compared the behaviour of single or multiple balloon expandable Wiktor stents, to balloon angioplasty alone or no intervention.

The acute effect of stenting of the 31 days old vein graft Scanning Electron microscopy revealed that except for the visible damage directly underneath the wires, no apparent damage to the vessel wall was inflicted by the implantation procedure as demonstrated by the microscopically normal endothelium. Due to the coil configuration of the stent, however, the luminal side was not smooth after the procedure (Figure 2A). The resulting flow disturbances might in turn induce thrombus deposition and persistent endothelial damage, which may both influence the process of intimal hyperplasia.

Angiography From the angiograms, the grafts were classified during the procedure qualitatively (by visual assessment) either as having only mild stenoses (later quantitatively analyzed as 32%, range 23-60%) in which case no further treatment was given, or as having severe stenoses (48%, range 19-70%) in which case they were assigned to either balloon angioplasty, single stenting or multiple stenting. Although some differences in angiographic minimal luminal diameter were observed between the intervention groups (see Figure 3A) these differences were not statistically significant. In these groups, angiography was again performed directly after the intervention. Comparison with the no-intervention group revealed that only in case of single stenting a minimal diameter was created that was similar (Figure 3B). During the four weeks of follow-up, a significant deterioration occurred in the mean MLD of all treatment groups. It seems, however, from the angiographic data of the grafts that remained patent throughout the study (Figures 3A and 3B) that for the single stent and balloon angioplasty group there are only two possible results in final outcome. Either the increase in MLD after intervention is maintained, or treatment results in total occlusion. Single stenting, however, does seem to be able to better maintain the procedural increase in MLD than balloon angioplasty alone (Figure 3B).

Histology and immunocytochemistry Human saphenous vein bypass grafts, studied within one months after surgery reveal minimal to mild fibrointimal hyperplasia. Some of this narrowing may well be preexisting since Thiene et al. [15] described that of 150 grossly "normal' veins, only 10 were totally free of microscopic phlebosclerosis. The porcine vein grafts from our study exhibited a more extensive fibrointimal hyperplasia. The high occlusion rate of 45% is likely to be due to overdistention induced injury and thrombosis superimposed on intimal hyperplasia, as indicated by haemosiderin deposits and neovascularization.
Following stent implantation, plasma proteins, platelets and leucocytes adhere to the metal wires. When compared to healthy, normal vessels, the thrombotic response to injury, tends to be more pronounced in vessels with a prior history of disease such as intimal thickening

[16] and might act in synergy with the stent.
From the histology of stented anastomotic sites, we observed that the intimal thickening was more pronounced in the grafts than in the adjacent "normal" carotid artery. Additionally, thrombus remnants which were still conspicuously present in the graft in close apposition with the stent wires, were cleared in the carotid artery within the same period. This indicates that either the thrombotic response to this type of injury is indeed more pronounced, or the organization of the thrombus is delayed or impaired in the graft. More likely is a combination of both since the incomplete incorporation of the stent in the vessel wall is indicative of a slow healing wound. Also histological examination of stents in older human vein grafts revealed the persistence of thrombus remnants [17].

Balloon angioplasty versus stenting with single or multiple endoprostheses In this model of early saphenous vein graft stenosis, balloon angioplasty and single stents resulted in comparable short-term patency rates and mean MLD. Preliminary, clinical results of placement of a self-expanding metal stent in older vein grafts suggest that this treatment has a more favourable clinical course when compared to balloon angioplasty [15]. The implantation of multiple stents, however, yielded significantly more luminal narrowing at follow-up in our experiments. Therefore, our results suggest that the implantation of multiple stents is not indicated in early bypass graft failure. Whether the same applies to late vein graft stenosis remains to be determined, but preliminary reports indicate that in humans, multiple stenting is indeed associated with an increased risk for restenosis [18].

Limitations of the study Several factors may limit the conclusions from this study: 1) The non-random distribution of grafts may have been an advantage for the "no intervention" group, because their initial diameter was larger than the others. The mean diameter of the grafts that remained patent throughout the study also indicate this to be an important parameter. Although some controversy still exists on the importance of the initial diameter, it was found that a small vessel diameter increased the risk of stent thrombosis, as well as small stent sizes [19].
2) Histological analysis showed that at two months inflammatory infiltrates were still present in the untreated grafts and especially at the anastomotic sites. The relatively early interventions, only one month after grafting, while healing was still ongoing, may have elicited a second more pronounced inflammatory process, which may have disturbed wound healing, and increased intimal and medial hyperplasia or even promoted (secondary) thrombotic occlusion. Better results can be expected if the intervention is delayed until healing of the vessel wall is completed.

CONCLUSIONS

The present study shows that:
Single stenting and balloon angioplasty seem to be able to maintain the increase in MLD during follow-up when considering patent grafts only, and in case of single stenting with less variability. Overall however, they do not decrease nor significantly improve patency (assessed by angiography) when stents are placed early (within 31 days) after grafting. Multiple stenting of porcine saphenous vein carotid bypass grafts, however, results in decreased patency. Additional antithrombotic therapy might have yielded better results.

Acknowledgements The authors sincerely wish to thank Robert van Bremen for his technical assistance and indispensable help during the experiments.

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Chapter 6

Histology after stenting of human saphenous vein bypass grafts. Observations from surgically excised grafts, 3 - 320 days after stent implantation.

Histology After Stenting of Human Saphenous Vein Bypass Grafts: Observations From Surgically Excised Grafts 3 to 320 Days After Stent Implantation

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Objectives. To gain insight into the mechanism of stenting in humans and its short- and long-term implications, we studied the vascular wall of saphenous vein aortocoronary bypass grafts after implantation of the Wallstent.

Background. The implantation of a stent in aortocoronary bypass grafts may provide an alternative solution for revascularization in patients who are poor candidates for reoperation. Because human histopathologic findings after stenting with the Wallstent have not previously been described in detail, we examined graft segments that were surgically retrieved from 10 patients (21 stents) at 3 days to 10 months after implantation of the stent.

Methods. The grafts were examined by a combination of the following techniques: light microscopy, immunocytochemistry and both scanning and transmission electron microscopy.

Results. Early observations revealed that large amounts of platelets and leukocytes adhered to the stent wires during the first few days. At 3 months, the wires were embedded in a layered new intimal thickening, consisting of smooth muscle cells in a collagenous matrix. In addition, foam cells were abundant near the wires. Extracellular lipids and cholesterol crystals were found after 6 months. Smooth muscle cells and extracellular matrix formed the predominant component of restenosis. This new intimal thickening was lined with endothelium, in some cases showing defect intercellular junctions and abnormal adherence of leukocytes and platelets as late as 10 months after implantation.

Conclusions. This type of stent is potentially thrombogenic and seems to be associated with extracellular lipid accumulation in venous aortocoronary bypass grafts.

(J Am Coll Cardiol 1993;21:45–54)

Coronary artery bypass graft surgery using autologous veins is a common treatment for atherosclerotic coronary artery disease. However, 12% of patients with grafts require a second revascularization procedure after 5 years (1), that in the case of repeat bypass surgery, is associated with increased morbidity and mortality and inferior long-term patency with respect to results of the first procedure (2). Moreover, a significant number of patients are poor candidates for a repeat operation (3). Percutaneous transluminal coronary angioplasty may provide an alternative to surgery for many of these patients. Although the initial success rate of this procedure is high (90%), the restenosis rate in bypass grafts is also high. Only 41% of patients are alive and event-free after 2 years (4). Furthermore, not all patients not eligible for surgical treatment, are candidates for balloon angioplasty (3).

The implantation of endovascular prostheses, also called stents, seems a promising new technique (5–8). The Thoraxcenter experience with the self-expanding Wallstent implanted in stenosed aortocoronary vein grafts now includes 69 patients with a total of 136 stents. Although the number of early complications in this high risk group of patients is considerable, the 34% rate of late restenosis compares favorably with that associated with angioplasty alone (8). From 10 patients with either early or late complications, we were able to obtain stented graft segments for histopathologic study that would provide a better understanding of the mechanism of stenting, as well as the short- and long-term effects of the procedure on diseased human vein grafts.

Methods

Patient characteristics. Between 1988 and 1990, a total of 136 stents (Wallstent) were implanted in aortocoronary vein grafts in 69 patients at the Thoraxcenter. All patients had severe symptoms and were poor candidates for repeat surgery or conventional balloon angioplasty for reasons such as

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Table 1. Characteristics of 10 Patients With Histopathologically Studied Stents

Pt No.	No. of Stents	Pre-PTCA	Stent Diameter (mm)	Duration of Implant (days)	Graft Age (yr)	Implant Reason	Retrieval Reason
1	3	Yes	4/4.5	3	10	UAP; primary; rescue	Bleeding
2	2/2	Yes	3.5/4	4/117	4	UAP; restenosis; elective	Occlusion
3	2	Yes	5.5/6	10	10	UAP; total occlusion; primary; elective	Occlusion
4	2	Yes	3.5	14	4	UAP; total occlusion (MI); primary; elective	Occlusion
5	1	?	?	85	8	Angina; rescue	Angina (other stenosis)
6	3	No	4	105	0.6	UAP; primary; elective	Restenosis
7	1/1	No/Yes	4.5/4.5	106/320	12	Primary/restenosis/both elective	Restenosis of primary lesion
8	2	Yes	5	184	6	Restenosis; elective	Restenosis
9	2	Yes	4	189	3	Primary stenosis	Restenosis
10	1	Yes	4.5	205	5	Restenosis; elective	Stenosis of stent ends

MI = myocardial infarction; Pt = patient; Pre-PTCA = angioplasty before stent implantation; UAP = unstable angina pectoris.

graft age (mean 83 months), lesion morphology or native coronary artery anatomy (3). Nine patients described here were from this group. One additional patient received a Gianturco-Roubin stent at the University of Michigan, Ann Arbor.

Graft segments were excised between 3 and 320 days after stent implantation during surgery for replacement of the stented bypass grafts (Table 1) and underwent pathologic analysis as approved by the Internal Research Board at the Thoraxcenter. Thrombosis within the stented segments or the need to interrupt aggressive anticoagulant therapy was an indication for surgical retrieval within the 1st 2 weeks. Restenosis within or adjacent to the stented segments was the indication for late reoperation.

Description of the stent. Wallstent endovascular prostheses (Schneider Europe AG) and the method of implantation have been described in detail (5). Briefly, the stent is a self-expanding, multifilament, woven mesh, constructed of 18 to 20 stainless steel wire filaments, each 70- to 90-μm wide. It is constrained in an elongated fashion on a delivery catheter covered with a removable plastic sleeve. The stent mounted on the delivery system is brought into place by using a standard over the wire technique. By withdrawing the sleeve, the stent is released and anchors itself against the vessel wall in an attempt to regain its original diameter.

Histopathologic analysis. The main histologic features of the vein grafts are summarized in Table 2. The surgically retrieved specimens were processed for light microscopy (all cases), immunocytochemistry (three cases), electron microscopy (seven cases) or a combination of these techniques. The grafts were briefly rinsed in 0.96% saline solution. For light microscopy and immunocytochemistry, the material was fixed in a phosphate-buffered solution containing 4% paraformaldehyde for ≥48 h. For electron microscopy, the material was fixed in a phosphate-buffered solution containing 4% paraformaldehyde and 1% glutaraldehyde for ≥48 h. Glutaraldehyde makes the specimen suitable for electron microscopy but renders it useless for immunocytochemical analysis.

After fixation, several 3- to 4-mm wide transverse sections were made, either of the whole vessel or, if electron microscopy was performed, from longitudinally cut vessels. The metal stent wires were removed by carefully pulling them out under a dissection microscope with a pair of fine tweezers. No fixed protocol was followed for studying the material.

Light microscopy. Paraffin-embedded tissue was stained with hematoxylin-azophloxine as a routine stain. Resorcin-fuchsin was used as an elastin stain; Goldner trichrome was used as a connective tissue stain. For Epon-embedded tissue, toluidine blue was used.

Immunocytochemical analysis. After rehydration, the sections were exposed to antibodies against smooth muscle cell-specific α-actin (mouse monoclonal antibody, Enzo Diagnostics), vimentin (rabbit polyclonal antibody, Eurodiagnostics) and desmin (mouse monoclonal antibody, Sanbio). Ulex europaeus (UEA-I, DAKO) was used as an endothelial cell marker. As a detecting reagent, horse radish peroxidase-labeled rabbit antimouse or goat antirabbit antibodies were used. Mayers hematoxylin was used as a counter stain.

Electron microscopy. After fixation, the material was rinsed and stored overnight in 0.1 mmol/liter of cacodylate buffer (pH 7.3). Postfixation was performed with a mixture of 50 mmol of $K_3[Fe\{CN\}_6]$ and 1% OsO_4 in 0.1 mmol/liter of cacodylate buffer (pH 7.2) for ≥4 to 6 h. After rinsing the specimen in 0.1 mmol/liter of cacodylate buffer, the material was dehydrated in a graded ethanol series.

For scanning electron microscopy, the specimen was critical point-dried in liquid carbon dioxide, mounted on a specimen table and sputter-coated with gold before examination in a Jeol-JSM-25 or 5200 scanning electron microscope.

For transmission electron microscopy, the material was

Table 2. Angiographic Data, Cholesterol Levels at the Time of Stent Implantation and Histologic Findings in the 10 Study Patients

Pt No.	QCA Pre-, Post-Stent and Follow-Up: Reference (mm)	Stent (mm)	MLD (mm)	Cholesterol Levels (mmol/liter)	Main Histologic Features
1	Rescue	No data	Rescue	4.8	Platelet and leukocyte adhesion to the stent
2	2.5 → 3.1 → 2.5	2.8 → 2.3	0.7 → 1.8 → 0.8	No data	Fibrous vein graft, thrombus
	3.4 → 4.8 → 2.5		1.2 → 2.7 → 0.8		
3	4.5 → 4.3 → 0	3.4 → 0	1.4 → 2.9 → 0	7.8; hypertriglyceridemia	Thrombus on atheromatous debris
	4.1 → 4.3 → 0	3.4 → 0	1.4 → 2.9 → 0		
4	0.0 → 2.7 → oc	2.6 → 0	0.0 → 2.4 → 0	4.5	Occlusive clot on atheromatous debris
5	No data	No data	No data	No data	Fibrous vein graft; limited new intimal thickening barely covering the stent; foam cells near stent wire
6	2.5 → 3.3 → 3.0	? → 2.7	1.2 → 2.4 → 2.2	5.1	Mostly limited new intimal thickening; fibrin remnants near stent wire
	2.5 → 3.3 → 3.6	? → 2.7	1.2 → 2.4 → 1.5		
	2.5 → 3.3 → 3.6	? → 2.7	1.2 → 2.4 → 1.5		
7	3.7 → 3.7 → 3.1	3.1 → 1.6	1.6 → 2.4 → 1.2	10.8	Vein graft with fibrous preexisting intimal thickening; new intimal thickening contains foam cells on top of stent wire; narrowing consists of smooth muscle cells and extracellular matrix
	4.2 → 4.3 → 4.5	3.8 → 4.2	1.9 → 3.7 → 4.2		
8	3.8 → 3.6 → 2.8	3.5 → 2.0	1.5 → 2.9 → 1.2	9.3	Fibrous vein graft; atheromatous plaque in new intimal thickening above the stent
	4.0 → 4.7 → 2.2	3.6 → 2.8	1.5 → 2.9 → 1.6		
9	2.8 → 2.8 → 3.1	2.6 → 2.7	0.8 → 2.1 → 1.2	No data	New intimal thickening consists of smooth muscle cells and extracellular matrix; fibrin near stent wires; recent thrombus
	2.7 → 3.2 → 3.2	3.2 → 2.4	0.8 → 2.7 → 0.4		
10	3.2 → 3.4 → 2.5	3.2 → 2.4	1.6 → 2.8 → 1.0	No data	Fibrous vein graft, atheromatous plaque in new intimal thickening above the stent; fibrin near stent wires

MLD = minimal lumen diameter; oc = occluded; Post = after; Pre = before; Reference = reference diameter calculated by the Quantitative Coronary Angiography (QCA) system; Stent = mean lumen diameter.

embedded in Epon. Ultrathin sections were cut with a diamond knife and collected on copper grids. Sections were stained with uranyl acetate and Reynold's lead citrate. Examination was performed in a Philips EM400.

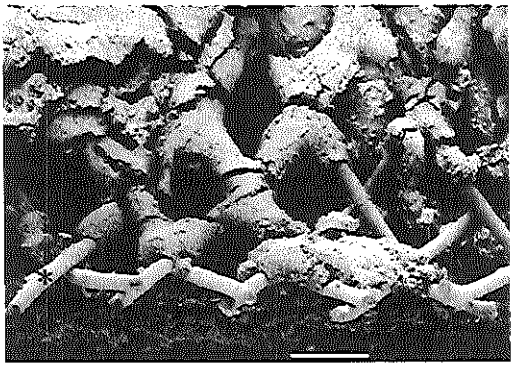
Results

Histologic findings 3 to 14 days after stent implantation. The earliest observation was 3 days after implantation of two stents <1 in. (<2.54 cm) apart, that were bridged several hours later by a third stent after ST segment elevation occurred and angiography revealed haziness (Tables 1 and 2, Fig. 1). Macroscopic examination showed no thrombotic mass, but scanning electron microscopy revealed significant amounts of leukocytes, platelets and fibrin adherent to the wires.

In Patients 2 to 4 (Tables 1 and 2), the grafts were resected because of acute thrombotic complications in the stented segments. Light microscopic analysis of the material from Patient 3 revealed that the proximal, nonstented graft segment (Fig. 2A) contained a preexistent circumferential layer resembling foam cells. However, the mechanical effect of stenting was clearly demonstrated in the adjacent stented segment (Fig. 2B), where the lumen diameter was enlarged. The stented segment further downstream (Fig. 2C) contained a classic atherosclerotic plaque with necrotic, lipid-rich cholesterol crystals, containing a core and a thin fibrous cap. This cap had been ruptured either during angioplasty or the stenting procedure (although a mechanical artifact cannot be excluded). The stent wires, however, neatly tacked the intimal flap against the vessel wall.

Histologic findings 3 months after stent implantation. In Patient 5 (Tables 1 and 2), scanning electron microscopy of the stent revealed complete covering with polygonal endo-

Figure 1. Patient 1. Scanning electron microscopy 3 days after stent implantation. Large deposits of leukocytes and platelets are found adherent to the wire mesh, especially at branching points. In some places, the vessel wall protruded through the wires into the lumen. *Indicates the stent wire. Bar = 500 μm.



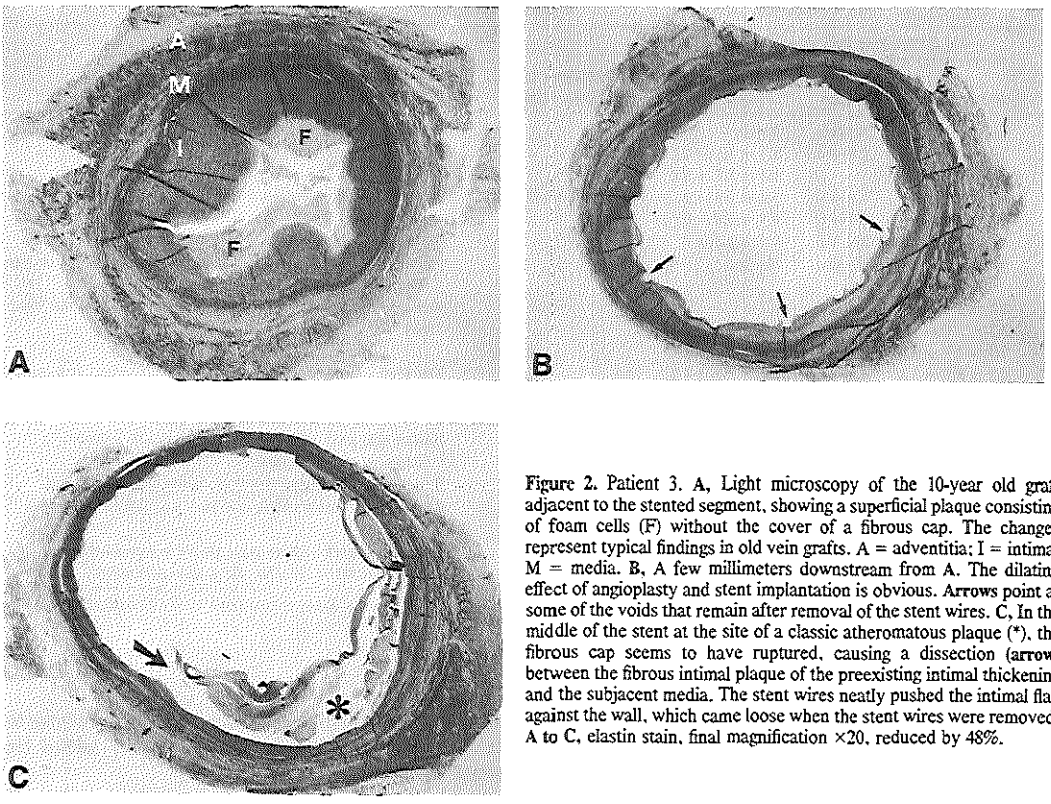


Figure 2. Patient 3. A, Light microscopy of the 10-year old graft adjacent to the stented segment, showing a superficial plaque consisting of foam cells (F) without the cover of a fibrous cap. The changes represent typical findings in old vein grafts. A = adventitia; I = intima; M = media. B, A few millimeters downstream from A. The dilating effect of angioplasty and stent implantation is obvious. Arrows point at some of the voids that remain after removal of the stent wires. C, In the middle of the stent at the site of a classic atheromatous plaque (*), the fibrous cap seems to have ruptured, causing a dissection (arrow) between the fibrous intimal plaque of the preexisting intimal thickening and the subjacent media. The stent wires neatly pushed the intimal flap against the wall, which came loose when the stent wires were removed. A to C, elastin stain, final magnification ×20, reduced by 48%.

thelial-like cells and some single missing cells. Some areas exhibited loose junctions between cells, as well as a ruffled surface (Fig. 3A). Leukocytes were also found adhering to the intact endothelium, a feature considered abnormal for mature endothelium. Light microscopy revealed a new intimal thickening, consisting of several layers of smooth muscle cells within the stented grafts.

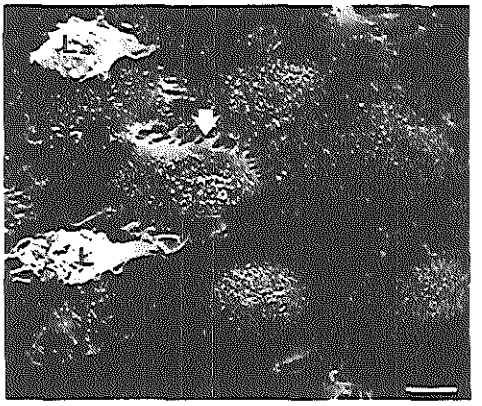
In a triangular area around the stent wires, we found various signs of the thrombotic event after stent implantation, consisting of fibrin (hematoxylin and azophloxin stain, not shown) in Patients 5 and 6 (Tables 1 and 2, Fig. 3B and C and 4) and abundant foam cells in Patient 7 (Fig. 3B and 5A). In this last patient (Case 7, Tables 1 and 2), the segment with the narrowest residual lumen contained a recent mural thrombus (Fig. 5B).

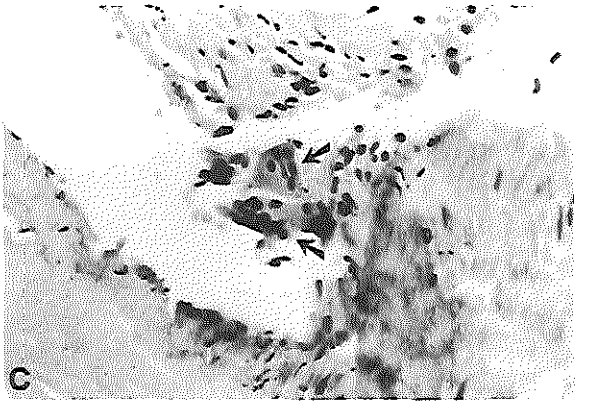
Histology 6 to 10 months after stent implantation. The second, nonrestenosed, stented graft segment in Patient 7 (Tables 1 and 2) resected 10 months after stent implantation was patent. Scanning electron microscopy revealed complete covering with endothelial cells, some with a protuberant appearance; leukocytes were also seen adhering in large numbers. Histopathology revealed a new intimal thickening similar to the nonstenosed normal areas in the earlier cases. Foam cells were found scattered in this layer but were abundant around the stent wires.

Patient 8 (Tables 1 and 2) had two closely situated stents resected 6 months after implantation. Restenosis had developed in the proximal end of both stents. Although the endothelial lining seemed intact, transmission electron microscopy showed focal leukocyte infiltration. The new intimal thickening consisted of smooth muscle cell-like cells that exhibited an abundance of cell organelles and bundles of myofilaments in a collagenous matrix. In addition to foam cells around the stent wires, we also observed extracellular lipids and cholesterol crystals similar to a classic atherosclerotic plaque.

Histologic examination of the tissue within two restenosed stents in Patient 9 (Tables 1 and 2) revealed dendritic-like cells within an abundant collagen-rich matrix. Fibrin deposits were found near the stent wires and a small recent mural thrombus was found at the lumen surface.

In Patient 10 (Tables 1 and 2), the graft was resected for recurrence of angina pectoris 7 months after stenting. Two months earlier, angioplasty was performed for restenosis of





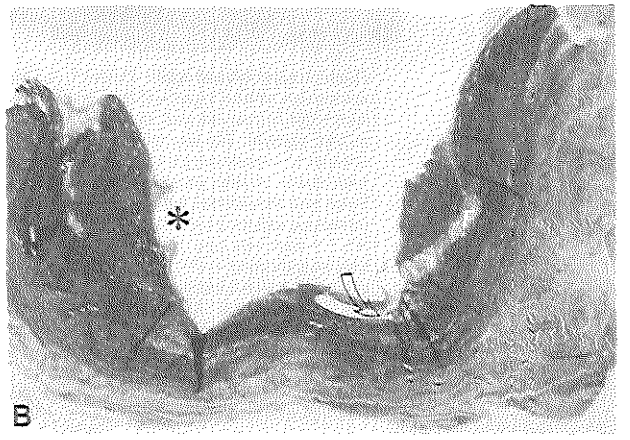


Figure 3. Patient 5. **A**, Scanning electron microscopy showing the endothelial lining, 3 months after stent implantation. Abnormal features seen include polygonal shape, loose intercellular junctions (**arrow**) and leukocyte adhesion (L). Bar = 5 μm. **B**, Light microscopy from a section proximal in the stent, showing fibrin remnants (**bright red**) around a stent wire void (*), probably deposited shortly after stent implantation. The slit-like appearance of the stent wire void (**arrow**) indicates that the wire is located near the areas with foam cells (F). A multinucleated giant cell (**arrow**) is in close contact with the stent wire. Fibrin Lendrum stain, final magnification ×40, reduced by 45%. **C**, Closer detail of **B**, showing the multinucleated giant cell (**arrows**). Hematoxylin-azophloxine stain, final magnification ×430, reduced by 35%.

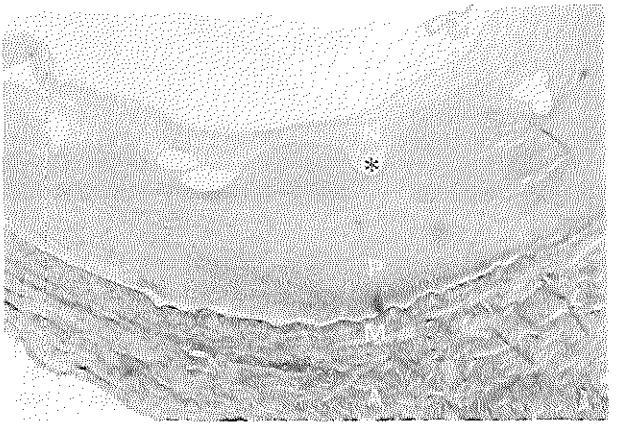
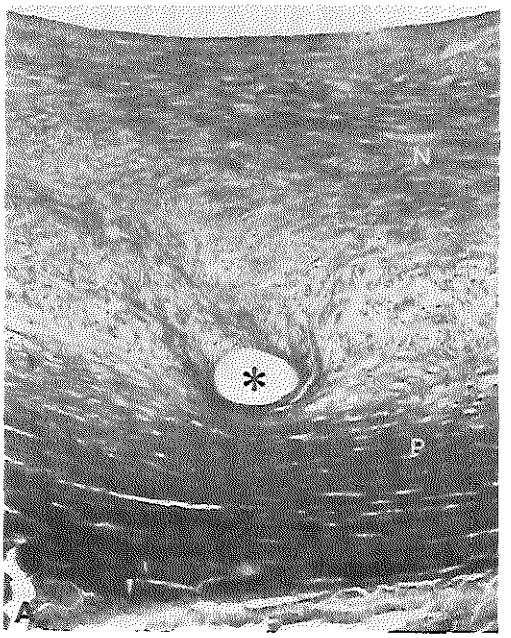


Figure 4. Patient 6. Light microscopy of a 6-month old graft obtained 3 months after stent implantation. Thrombus remnants (yellow areas) are still conspicuously present around the stent wire voids (*) and clearly demarcate the border between preexisting intimal thickening (P) and new intimal thickening (N). A = adventitia; M = media. Elastin stain, final magnification ×40.



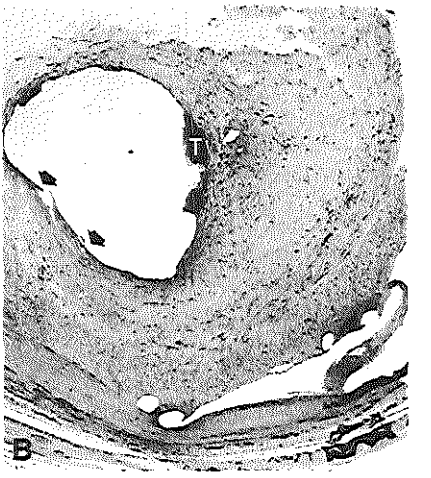


Figure 5 (upper left and right). Patient 7. A, Light microscopy within 3 months of stent implantation shows the 12-year old graft narrowed within the stented segment. The new intimal thickening (N) consists of smooth muscle cells within a collagenous matrix. A large amount of foam cells (F) is found near the junction between the new intimal thickening and the preexisting intimal thickening (P). * = stent wire void. Goldner trichrome stain, final magnification ×107. B, At the site with the narrowest residual lumen, a recent mural thrombus (T) was found. Although the new intimal thickening nearest to the lumen is relatively acellular (**arrows**) near the thrombus, a region with increased cellularity and neovascularization is observed. Toluidin blue stain, final magnification ×40, reduced by 35%.

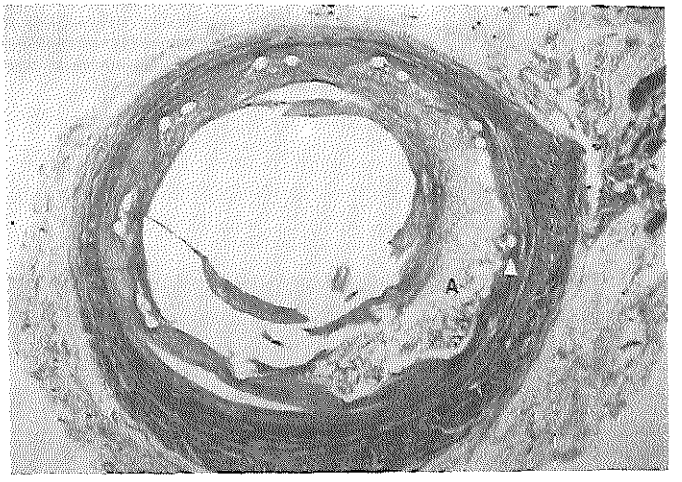


Figure 6 (lower left). Patient 10. Light microscopy 6 months after stent implantation. Proximal in the stented graft segment, an atheromatous plaque (A) is located on top of the stent wires (*). This micrograph, however, does not represent the minimal lumen diameter. Fibrin remnants (**arrowhead**) partly surround the wires. Goldner trichrome stain, final magnification ×21, reduced by 35%.

the stent ends. The proximal stent segment located near the ostium of the graft was not excised. As in Patient 8, histopathologic study revealed a localized atherosclerotic plaque at the lumen side of the stent wires (Fig. 6). The distal stent end revealed a long dissection within the new fibrous intimal thickening.

Immunocytochemistry. Immunocytochemistry with positive staining for the lectin Ulex Europaeus confirmed that the new intimal thickening of three patients (Cases 6, 9 and 10) was covered by endothelium. Analysis with smooth muscle cell-specific anti-alpha-actin antibodies confirmed the presence of smooth muscle cells in the new intimal

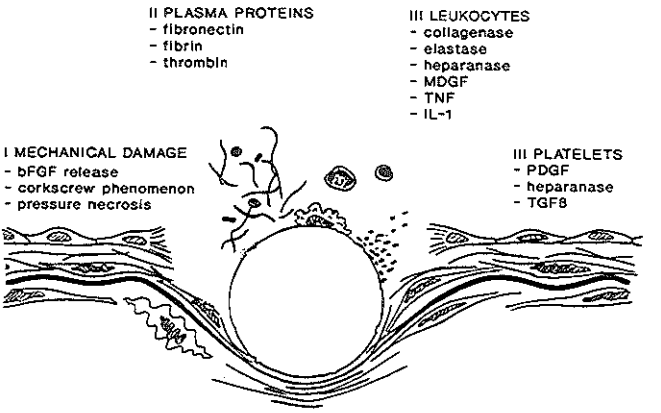


Figure 7. Early events after stenting. The first event, mechanical (balloon- and stent-related) damage, is mainly inflicted during the implantation procedure and induces the release of basic fibroblast growth factor (bFGF) from damaged cells and matrix. As soon as blood flow is restored, plasma proteins adhere to the stent and damaged areas, followed by platelets and then leukocytes, an important source of growth factors, growth modulators, cytokines and enzymes. Surface irregularities to which macrophages often adhere can trap air nuclei, which are highly thrombogenic. IL-1 = interleukin-1; MDGF = macrophage-derived growth factor; PDGF = platelet-derived growth factor; TGFβ = transforming growth factor β; TNF = tumor necrosis factor.

thickening. Analysis with anti-vimentin (synthetic smooth muscle cells) and anti-desmin (contractile smooth muscle cells) antibodies gave an indication of their phenotype. Vimentin-positive cells were especially abundant near the stent wires, whereas desmin-positive cells were found mainly in the media.

Discussion

The implantation of endovascular stents in aortocoronary bypass grafts may provide an alternative solution for revascularization in patients who are poor candidates for reoperation (3). Our analysis of stented grafts resected because of stent failure or a nonstent-related complication revealed that large amounts of platelets and leukocytes adhere to the metal stent wires, during the first days after implantation and despite extensive anticoagulation, indicating that this type of stent is potentially thrombogenic. We also observed that the stent wires exert considerable pressure on the vessel wall and thereby might be able to rupture the sometimes thin fibrous cap covering atherosclerotic plaque, a possible mechanism of acute or subacute occlusion several days after an initially successful procedure.

Within 3 months, the stents were completely embedded in the vessel wall and were covered by polygonal bulging endothelium, showing leukocyte adhesion. Near the stent wires, large numbers of foam cells and fibrin deposits were found.

Between 6 and 10 months after stenting the endothelium still appeared abnormal. Large extracellular lipid deposits containing cholesterol crystals and a necrotic core were observed near the stent wires.

Tissue that narrowed the vessels always consisted of smooth muscle cells within an extensive extracellular matrix.

To provide a framework for a better understanding of the mechanism of stenting and its short- and long-term implications, we constructed a scheme (Fig. 7 and 8) describing the sequence of events that we believe take place during and after stenting.

Mechanical Damage to the Vessel Wall

Acute injury (Fig. 7). Implantation of a stent is accompanied by damage to the endothelial lining and stretching of the vessel wall. The extent of acute injury depends on several factors, such as stent design, means of delivery, lesion morphology and additional balloon angioplasty. Stent size relative to the receiving vessel (9) determines the extent of stretching, whereas lesion morphology determines which part of the lesion or "normal" wall is stretched.

Chronic injury. The stent is also a source of constant injury. This is especially true of the self-expanding Wallstent, which exerts an uninterrupted radial force on the vessel wall (10). Oversizing this stent by >0.7 mm relative to the reference diameter was found to be associated with restenosis in both native coronary arteries and venous bypass grafts (11).

We have also studied pig coronary arteries 1 and 4 weeks after implantation with the balloon-expandable Wiktor stent. When grading the damage according to Schwartz et al. (12), we found that significantly more damage was observed after 4 weeks. Movement of a rigid implant within the vessel wall could cause this late damage (13). An alternative explanation is local weakening of the media underneath the wire as a result of reparative processes, which could be aggravated by vasospasm.

Injury and growth factors. As shown by Fingerle et al. (14), the early proliferative response of smooth muscle cells to injury occurs even in the absence of platelets. Thus, growth factors other than those derived from platelets play a major role in the early proliferative response.

A possible incentive for cell proliferation after injury is the release of endogenous pools of growth factors from vascular cells, such as basic fibroblast growth factor (Fig. 7) from endothelial cells and smooth muscle cells, which are

deposited at least by endothelial cells in the extracellular matrix (15). Until now, only endothelial cells are known to release basic fibroblast growth factor when mechanically damaged, but it has been shown that basic fibroblast growth factor is a potent mitogen for smooth muscle cells (16,17).

Thrombotic Response to Injury and Foreign Material

The first event after stent placement is the deposition of plasma proteins (such as fibronectin and fibrinogen) on the stent surface and injured areas (Fig. 7). This process—called surface conditioning—is followed by attachment of platelets and subsequently the growth of platelet- and leukocyte-rich thrombi (18), as illustrated by Patient 1. This thrombotic response to injury tends to be more pronounced in vessels affected by intimal thickening or atherosclerosis (19).

The thrombotic response to the stent is further determined by three important surface variables: surface texture or roughness, surface charge or electrochemical potentials and surface chemistry. A rough surface may retain small air bubbles (air nuclei), which will turn a basically thromboresistant surface into a thrombogenic surface. Avoiding air nuclei (denucleation) is probably a more important factor in the biologic response than surface charge and chemistry. The latter two variables predominantly determine which proteins adsorb to the surface (18,20). However, it is unknown how plasma proteins influence tissue proliferation.

Reconstruction of the Vessel Wall

Migration and proliferation of smooth muscle cells. Within several days after stent implantation, the thrombus is colonized by smooth muscle cells. The source of these smooth muscle cells is not known. They might migrate from the media or plaque on which the stent was placed, especially at sites of internal elastic membrane rupture (Fig. 8), or through gaps in the internal elastic membrane widened by excessive stretch or enzymatic degradation by macrophages. An alternative origin of smooth muscle cells is the subendothelial space adjacent to the injured site. In normal porcine coronary arteries (21), thrombus is cleared away within a few weeks after stenting, but in a porcine vein graft model (22) and in the present study, thrombus remnants as a nidus for smooth muscle cell colonization remain associated with the stents for much longer.

Endothelial regeneration. In young pigs, the stent is completely endothelialized within 1 week (21). In humans, it is unknown how long it takes before the endothelium is regenerated, but at 3 months, the process seems complete. Up to 10 months after the intervention, however, endothelial cells revealed a polygonal shape, prominent bulging nuclei and leukocyte adhesion. These features are commonly observed on endothelium covering atherosclerotic plaque (23) and are perhaps a general feature of these grafts, extending well beyond the stented segments.

There is experimental and clinical evidence (24,25) that endothelium can be dysfunctional for weeks after balloon denudation. The prolonged presence of thrombus elements may form a constant source of thrombin that is protected from degradation (26,27) and cytokines. The latter inhibit the expression of the thrombin receptor thrombomodulin and thrombin-stimulated secretion of tissue plasminogen activator and protein C activation. This can turn the anticoagulant endothelial surface into a procoagulant surface (28,29). The presence of small thrombi as seen in Patient 7 (Fig. 5B) may be an example of this defect.

Reconstruction and growth factors. Although we did not study growth factors in our specimens, it is known that wound repair after injury is not a simple process but rather a complex interaction among cytokines, cells and extracellular matrix (30). Damaged endothelium and macrophages are known to release basic fibroblast growth factor (16,31). Macrophages and platelets can release the enzyme heparanase (32), which degrades (subendothelial) extracellular matrix, thereby releasing more basic fibroblast growth factor. This in turn induces a phenotypic change in smooth muscle cells that renders them more susceptible to mitogens and atherogenic stimuli (15). We hypothesize that constant stent-related injury may induce a proliferative response (33,34) by both injuring the extracellular matrix and damaging the cell and subsequently releasing intracellular growth factor pools.

From Reconstruction to Lumen Obstruction (Fig. 9)

Within approximately 3 months, the prosthesis is embedded in the vessel wall and covered by endothelium. Has an end stage now been reached? Some of our observations indicate that this question may not be a rhetorical one. All grafts we examined, both restenosed and nonrestenosed stented segments, revealed considerable adhesion of leukocytes to the endothelial layer. This suggests an ongoing atherosclerotic process, influenced perhaps by an immune-mediated hypersensitivity reaction (35) or chronic endothelial dysfunction (24,25), or both, which in Patient 7 may have caused repeated thrombus deposition and ultimately narrowing of the vessel (Fig. 5B).

It is likely that vascular injury of any cause will elicit basically the same healing process (36). Stent implantation, however, may be unique because a persistent foreign body is introduced in the vessel wall. The presence of multinucleated giant cells as found in Patient 5 shows that a "foreign body reaction" to the stent is indeed induced. Macrophage giant cells in vitro survive only a few days unless replenished by "fresh" monocytes. The presence of multinucleated giant cells long after implantation therefore implies continuous division of macrophages at the implant site or an influx of circulating monocytes, or both (13).

In almost every case, we found large accumulations of foam cells or extracellular lipids on top of the stent. This might indicate preexisting atherosclerotic changes, which

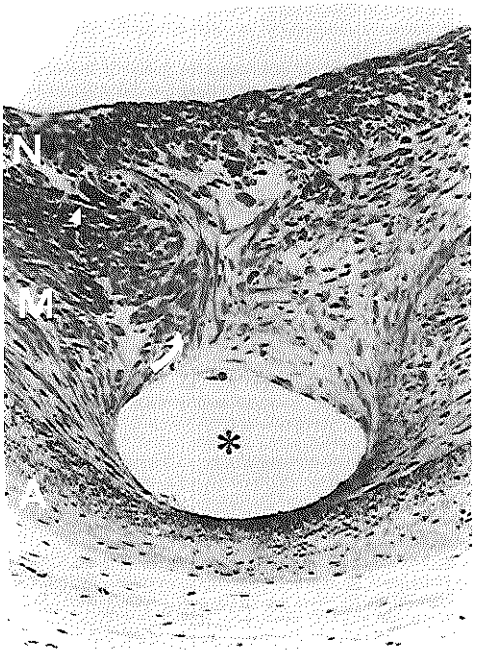


Figure 8. Medtronic Wiktor coronary stent 4 weeks after stenting of a porcine coronary artery. Geisers of smooth muscle cells seem to erupt from the media at the site of the stent wire (**curved arrow**). A = adventitia; M = media; N = new intimal thickening; * = stent wire void; **arrowhead** = lamina elastica interna.

commonly occur in old vein grafts, or we are dealing with a phenomenon induced by the stent. In case of preexisting atherosclerosis, the stent wires may have gone through superficial foam cell accumulation or even lacerated the fibrous cap on classic atheromatous plaques. Superimposed healing might create the suggestion of stent-related atheroma accumulation. The option of stent-induced atheroma accumulation, however, could be explained by monocyte adhesion during stent implantation in combination with thrombin-activated platelets, which are potent inducers of macrophage foam cell formation (37). Late adhesion and migration of leukocytes might add to this. Extracellular lipid pools might arise from macrophages that have reached the end of their life span, thereby releasing their contents in the vicinity of the stent.

In addition, the presence of fibrin near the stent wires deep in the atheromatous plaque indicates that the stent was in contact with flowing blood for a considerable amount of time and points in the direction of delayed rupture of atheromatous plaques (that is, a preexisting lesion) or accelerated atheroma accumulation (that is, a new lesion).

Conclusions

Stenting of old stenotic bypass grafts may provide an attractive alternative to repeat bypass surgery or balloon angioplasty in selected cases. The main histologic findings in this study are that considerable acute thrombotic responses take place, even in a case considered to be without thrombotic complication. Later on, abnormalities after vessel wall reconstruction appear chronic in nature and consist of layers of smooth muscle cells within an extensive collagenous matrix, enhanced white blood cell adhesion to the endothe-

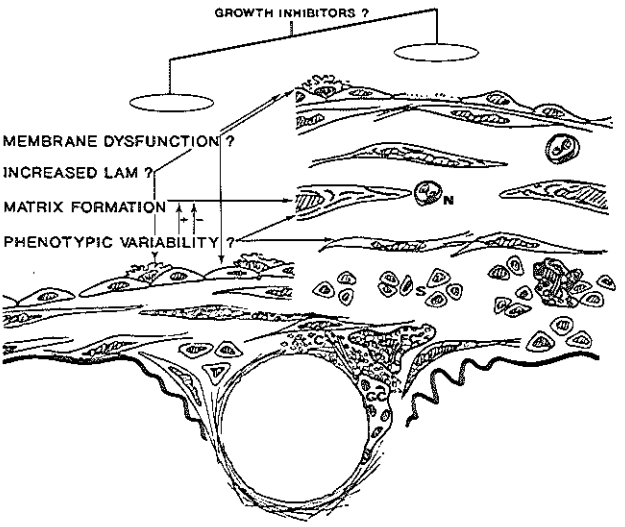


Figure 9. The chronic phase after stenting. During this phase, growth promoters and inhibitors may be out of balance, resulting in smooth muscle cell phenotypic variability, excessive matrix production and finally hyperplasia, whereas at the level of the endothelium, membrane dysfunction perhaps results in increased leukocyte adhesion, thrombogenicity and lipid infiltration. C = cholesterol crystals; FC = foam cells; GC = giant cells; LAM = leukocyte adhesion molecules; N = neocapillaries; S = smooth muscle cell in a longitudinal orientation.

lium and aggregates of foam cells and extracellular lipid accumulation. Although reconstruction of the vessel wall after arterial injury probably follows a uniform pattern, some of our observations may be unique to coronary stenting.

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Chapter 7

Stenting of coronary arteries: Has a modern Pandora's box been opened?

NEW TECHNOLOGY

Stenting of Coronary Arteries: Has a Modern Pandora's Box Been Opened?

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Interventional cardiology has recently witnessed the growth of several alternatives to percutaneous transluminal angioplasty, including coronary stenting. Although stenting appears to be useful in treating abrupt closure after coronary angioplasty, its effectiveness in limiting the complex processes responsible for late restenosis is much less certain. Pathologic examination of stented human saphenous bypass grafts shows extensive deposits of platelets, fibrin and leukocytes along the stent wires within the 1st week and formation of a neointima of variable thickness after 3 months without evidence of foreign body reaction. The long-term effects of continuous barotrauma induced by the expanded stent remain unknown. It is difficult to assess the relative merits of the new devices, but stenting has several theoretic advantages. It seems less disruptive to the underlying architecture of the vessel wall and enjoys favorable theoretic and effective expansion ratios. Widespread clinical acceptance for stenting will depend on demonstrating that its safety, efficacy and cost efficiency are superior to those of balloon angioplasty.

(J Am Coll Cardiol 1991;17:143B–54B)

The introduction of coronary balloon angioplasty by Andreas Gruentzig (1) in 1977 provided the stimulus for rapid technologic growth in the field of interventional cardiology. This development has produced several new devices designed to ablate coronary artery narrowings, recanalize occluded vessels and prevent restenosis. It is difficult to evaluate the relative merits of each intervention and to define their roles in clinical cardiology. In applying this new technology, cardiologists have limited their concern to the technical and procedural aspects, while sometimes overlooking the complex biologic and physiologic mechanisms of atherosclerosis, and in particular of the restenosis process. In achieving the perceived benefit of therapeutic intervention with these devices, the vessel wall is subjected to thermal and mechanical insults that may have hidden long-term consequences. An example is the restenosis process, which has been iatrogenically induced in tens of thousands of patients.

One of these newer developments has been the use of endoluminal vascular prostheses, although the original concept of intravascular stenting precedes the introduction of coronary artery interventional cardiology by many years. Since the original description of Dotter's tubular coil spring stent (2), many variants of the original concept have been introduced, including thermal shape memory alloy stents (3–6), steel spirals (7), stainless steel mesh stents (8–11), slotted stainless steel tubes (12–15), zigzag stents (16–18), U-configuration bends (19), interdigitating coils (20–22), tantalum helical coil stents (23), knitted tantalum wire stents (24,25), removable, metallic mesh stents (26) and synthetic polymeric and biodegradable stents (27). These various devices differ greatly in their fundamental geometry (tube, mesh or single wire), composition (metal or plastic) and mechanical behavior (active or passive expansion). Furthermore, there are a variety of subtle differences that may be important in themselves, such as thickness of filaments, alloy composition, electrostatic behavior and biocompatible or therapeutic coatings.

More than 7 years have passed since the first clinical report (8) of successful coronary stent implantations. Although the current world experience has now exceeded 1,000 stent implantations, the clinical indications and applications of this prosthesis remain undetermined and even experienced investigators are uncertain on these issues. The current status of the coronary stent parallels that of other recently introduced technologic advances, including laser angioplasty and atherectomy (directional and nondirectional types). This raises a fundamental question: have we been unable to realize the full potential of these newer devices because of our limited understanding of the underlying biologic interactions, particularly those responsible for restenosis? In this review, we address several relevant issues based on our experience in the evaluation of various coronary stents.

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Rationale for Stenting an Atherosclerotic Stenosis During or After Balloon Dilation

Short-term consideration: abrupt closure. Abrupt vessel closure after angioplasty occurs in 2% to 11% of procedures (28–31). Intimal flaps induced by the arterial injury can disrupt flow by partially or completely occluding the lumen. Sluggish anterograde flow and the exposure of media to procoagulant blood-borne elements are potent thrombogenic stimuli that further contribute to the process. The coronary stent, by acting as a splint, can physically contain the protruding obstructive flap and maintain flow as well as possibly prevent distal embolization of macroscopic debris originating from the plaque or flap (32). This scaffolding function appears to be a property common to both balloon and self-expanding stenting prostheses. Angiographic studies (10) after stent implantation have shown that the self-expanding Wallstent has a smoothing effect that reduces calculated poiseuille and turbulent contributions to flow resistance. Several reports have documented successful deployment of the various types of stents in the "bail-out" situation when the presence of intimal dissection has led to a poor and even critical hemodynamic result (33,34).

Long-term considerations: restenosis. Restenosis remains the major limitation of coronary angioplasty. Despite a lack of uniformity of definition, several angiographic follow-up studies have documented a 20% to 40% incidence in the 1st 6 months after angioplasty (35–38). Restenosis has been defined angiographically as a significant deterioration in the luminal diameter of a lesion that had previously been successfully dilated, and does not necessarily indicate a common pathologic substrate. Diverse histologic processes may be responsible for restenosis depending on the time interval since angioplasty.

Early restenosis. Restenosis has been documented in up to 11% of lesions as early as 1 to 4 days after coronary angioplasty (38–40). It is believed that the early cases of angiographic worsening are a result of several processes, including elastic recoil, vasospasm or platelet-fibrin thrombi, or combinations. This time interval is too brief for significant fibrointimal hyperplasia to have occurred, for several reasons. Pathologic studies (41–43) of vessels retrieved <10 days after angioplasty have not shown any significant intimal hyperplasia. Animal experiments (44,45) in carotid arteries of rats have demonstrated that smooth muscle cell migration into the intima begins only at 4 days and maximal intimal smooth muscle cell proliferation is not noted before 7 days after balloon endothelial denudation. Furthermore, cell cultures of medial smooth muscle cells (46) have shown that the modulation of phenotype from the quiescent, contractile state (typical of normal medial smooth muscle cells) to a metabolically active, synthetic state occurs only after 6 to 7 days. Smooth muscle cells obtained from intimal thickenings phenotypically resemble these synthetic-type smooth muscle cells observed in culture and share a common cytoskeleton protein profile that differs from typical medial smooth muscle cells (47,48).

The stent and elastic recoil. The significance of elastic recoil has been demonstrated acutely during angioplasty. In a study of 151 dilated segments, the minimal luminal cross-sectional area before angioplasty was 1.1 ± 0.9 mm^2 (49). Immediately after the procedure the cross-sectional area of the dilated vessel was 2.8 ± 1.4 mm^2. Elastic recoil, defined as the difference between the balloon cross-sectional area (5.2 ± 1.6 mm^2) and the vessel area after angioplasty, was calculated to be 2.4 ± 1.4 mm^2, which is almost 50% of the cross-sectional area of the fully inflated balloon. In an angiographic study of the initial 117 stent implants, we demonstrated that the self-expanding Wallstent mitigates the effects of elastic recoil. Stenting immediately improved the minimal cross-sectional area from 3.0 ± 1.2 mm^2 after angioplasty to 5.5 ± 2.7 mm^2. In a subgroup of patients with angiography 24 hours later, the stent continued to expand and increased the cross-sectional area to 6.8 ± 4.4 mm^2 (Serruys et al., unpublished observations).

Late restenosis. Two processes have been implicated in the development of late restenosis. In some cases it has been attributed to the organization and fibrous conversion of platelet-fibrin thrombi that form at the site of intimal damage. However, a more important mechanism appears to be marked cellular proliferation within the vessel wall that is stimulated by complex interactions between platelets adherent to the damaged intima, macrophages, endothelial cells and medial smooth muscle cells. Pathologically, late restenosis is characterized by an aggressive proliferation of smooth muscle cells that presumably have migrated from the media into the intima, resulting in a variable degree of luminal narrowing (43,50). Immunoperoxidase staining of the cellular component of this fibrointimal tissue has identified the characteristic cytoskeleton proteins of medial smooth muscle cells—alpha actin, desmin and vimentin—confirming the origin of the cells responsible for this growth.

What Causes Smooth Muscle Cell Proliferation?

Abnormal smooth muscle cell proliferation is an intricate process that is only partially understood. Animal models have revealed that balloon denudation in arteries will stimulate a sequence of events if either of two conditions is present: 1) extensive endothelial denudation, or 2) significant medial smooth muscle cell injury.

The pioneering work of Reidy et al. (44,45) showed that significant intimal hyperplasia occurred after balloon injury in rat carotid arteries that resulted in the loss of up to 25% of the vessel wall deoxyribonucleic acid (DNA). This loss reflects widespread medial smooth muscle cell injury, since endothelial cell loss alone could not account for such a major change in DNA content. Later, more sophisticated techniques of vessel wall injury were used (51), which localized

damage to the endothelium, sparing the subendothelium and medial layers. Subsequently, intimal thickenings developed only in regions of the vessels that were not re-covered with endothelium after 7 days. These studies suggested that smooth muscle cell proliferation and migration are separately controlled processes, because some areas of rapid endothelial regrowth contained increased numbers of medial smooth muscle cells without a corresponding increase in intimal thickness. A separate series of autoradiographic experiments (52,53) showed that only 50% of intimal smooth muscle cells are capable of proliferation, supporting this concept.

Platelet-derived growth factor. Several mitogens have been implicated in the stimulation of smooth muscle cells. Platelet-derived growth factor, the most intensively studied factor, is a dimer compound composed of two homologous polypeptide chains (A and B) that are disulfide bonded (54). Although platelet-derived growth factor was originally isolated from platelets, further study has confirmed that it is released from several different cells, including vascular endothelium, macrophages and even activated smooth muscle cells, perhaps explaining why smooth muscle cells continue to proliferate long after the initial platelet-vessel wall interaction (55–58).

The binding of platelet-derived growth factor to its receptor initiates a complex cascade of signal transduction within the cytoplasm and ultimately into the nucleus of the smooth muscle cell, resulting in cell division and protein synthesis. Although these pathways have not been elucidated fully, important steps include the platelet-derived growth factor receptor-mediated phosphorylation of tyrosine kinase and activation of phospholipase C, which subsequently generates two important second messengers, diacylglycerol and inositol triphosphate (59,60). The platelet-derived growth factor receptor and both its chains have been sequenced and it is now possible to clone platelet-derived growth factor with recombinant technology. Monoclonal antibodies against both chains of platelet-derived growth factor and its receptor have also been produced. The gene that codes for the B chain mRNA of platelet-derived growth factor is c-sis, which is the cellular counterpart to the v-sis gene of the simian sarcoma virus. An intriguing connection between neoplasia and atherosclerotic lesions is the demonstration of an active human oncogene in atherosclerotic plaques (61). Cultured mouse fibroblast NIH 3T3 cells have been transformed with transfected DNA from these plaques (61). These transformed cells have established slow-growing tumors in "nude" mice.

Other growth factors. Other important growth factors that have been related to restenosis include interleukin-1 (IL-1), fibroblast growth factor, colony stimulating factor, epidermal growth factor, insulin-like growth factor (somatomedins), endothelin and serotonin. The relative influence of the individual factors and possible interactions are largely unknown and indicate our limited understanding of the entire process.

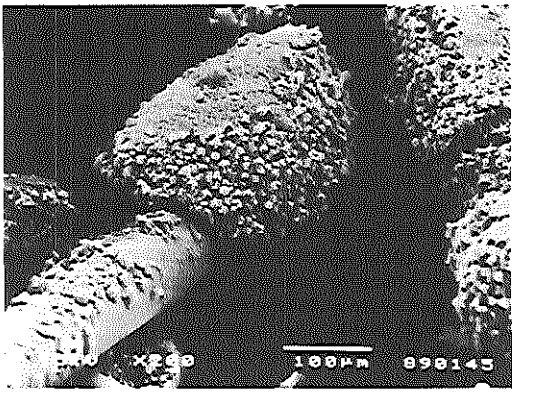


Figure 1. Scanning electron micrograph of a stented human saphenous vein bypass graft removed 3 days after implantation. Extensive deposits of platelets, leukocytes and fibrin are evident on this detail of the stent filaments.

Pathology of Restenosis: After Coronary Angioplasty and Coronary Stenting

Although autopsy reports of late follow-up angioplasty cases (3 to 20 months) are limited, the general consensus is that the characteristic features of restenosis are smooth muscle cell hyperplasia and a variable amount of extracellular matrix and fibrosis depending on the time elapsed since angioplasty. In addition to data from chronic animal studies, stented venous bypass grafts have been retrieved from several patients for analysis. Although the extent of intimal hyperplasia is similar after stenting to postangioplasty examination, several histologic features appeared to be unique to coronary stents.

Pathologic and histologic features. In human saphenous vein bypass grafts and porcine coronary arteries retrieved 3 to 7 days after stent implantation, extensive deposits of platelets, fibrin and leukocytes are observed along the stent wires (Fig. 1). In the pig, the stent wires become embedded in the vessel wall and are covered with a neointima within 7 days. This neointima consists of organizing thrombus directly adjacent to the wire and several layers of smooth muscle cells along the luminal surface (Fig. 2). Scanning electron microscopy has confirmed complete endothelialization.

At 4 weeks in porcine coronary arteries, few traces remain of the initial platelet-fibrin thrombus, which is represented by a few erythrocytes, leukocytes and lipid-laden foam cells that are interspersed in a disorganized fibrocellular layer (Fig. 3). At the luminal side two distinct layers of smooth muscle cells are present, one in a circular orientation immediately below the endothelium and a deeper layer in a longitudinal orientation.

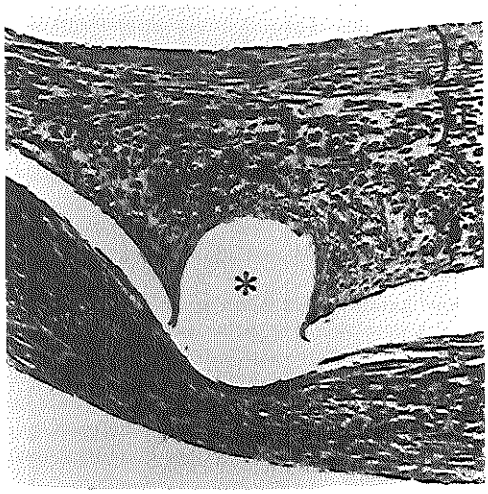
After 3 months, a more extensive neointima forms in the

Figure 2. Light micrograph of a stented porcine femoral artery 7 days after implantation. The voids (*) represent the sites of the 70 μm diameter stent wires, which have been removed. There is a disorganized layer of neointima (N) on the luminal aspect of the stent wire, containing smooth muscle cells, trapped red blood cells and fibrin. Above the disorganized layer is an organized neointima that contains smooth muscle cells covered by endothelium. The internal elastic lamina is interrupted at the left (**arrow**). A = adventitia; M = media.

porcine coronary artery with only a small area adjacent to the stent wire containing leukocytes and cellular debris (the so-called "Bermuda triangle") (Fig. 4). In human saphenous vein bypass grafts removed 3 to 10 months after the stenting procedure, the amount of neointima which develops is comparable with the amount of neointima in the porcine coronary artery, but the neointima in humans borders on the old atherosclerotic plaque (Fig. 5). At the junction between old plaque and recent neointima, abundant foam cells and extracellular lipid deposits are found within the new neointima in addition to extensive extracellular matrix production (Fig. 6).

Mechanisms. The causes and possible relations between these early and late histologic features are unknown. Two factors may be important. First, the regenerated endothelium that covers the stented segment may be dysfunctional and thus permit abnormal and excessive lipid infiltration and macrophage penetration across the endothelial barrier. Scanning electron microscopy of the endothelial lining has indicated an irregular, raised endothelial surface in lieu of the normal smooth covering (Fig. 7) although no permeability to Evan's blue dye was demonstrated in stented porcine arteries after 3 months (Van der Giessen et al., unpublished observations). Second, important chemotactic substances may be released by the cellular debris trapped in the tissue adjacent to the stent wires. This area appears to persist late after stenting for several reasons, including continued damage from direct pressure necrosis and its deeper location in the vessel wall, which isolates it from laminar flow patterns predominating on the luminal aspect of the stent wires. Striking similarities exist between the biology of stented vessels at 3 months and chronic atherosclerotic lesions, namely, proliferation of smooth muscle cells, large amounts of connective tissue matrix including collagen, elastin and proteoglycans, and lipid accumulation in the form of foam cells (smooth muscle cells and macrophages), and extracellular deposits. The natural history of these post-stent lesions has not yet been determined.

Figure 3. Light micrograph of a porcine coronary artery 4 weeks after implantation, showing the remnants of the initial thrombus, which now contains a few erythrocytes, leukocytes and lipid-laden foam cells that are interspersed in a disorganized fibrocellular layer. At the luminal side, two distinct layers of smooth muscle cells are present, one in a circular orientation (C) immediately below the endothelium, and the other a deeper layer in a longitudinal orientation (L). (*) = void representing the site of a removed 127 μm diameter stent wire.



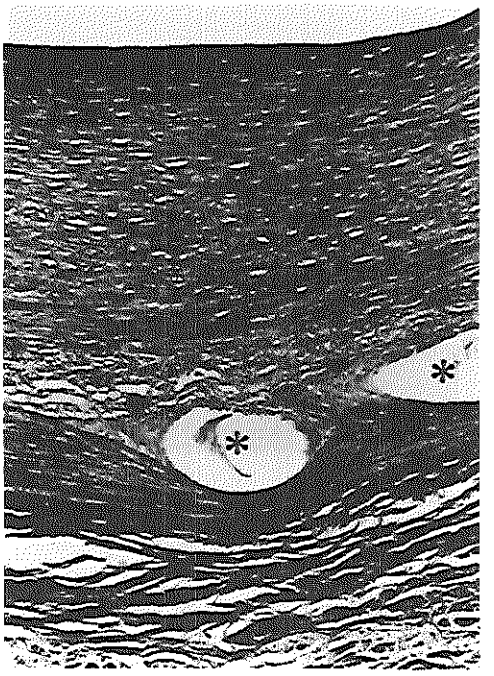


Figure 4. Light micrograph of a stented porcine artery 3 months after implantation. A more extensive neointima has formed, with only two small areas adjacent to the stent wire containing leukocytes and cellular debris (arrows). (*) = void representing the site of a removed 70 μm diameter stent wire.

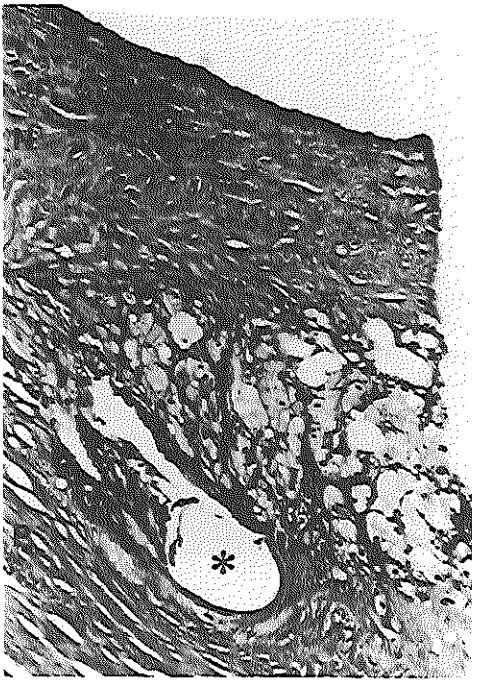


Figure 5. Light micrograph of a human saphenous vein bypass graft removed 10 months after stent implantation. A prominent neointima (N) has formed and borders on the old atherosclerotic plaque (P). At the junction between the old plaque and the recent neointima, abundant foam cells are found in the new neointima. (*) = void representing the site of a removed 70 μm diameter stent wire.

Stenting and Hyperplasia

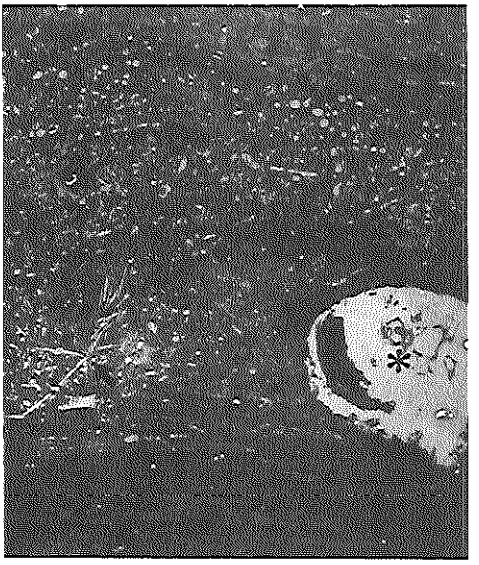
Role of stenting in hyperplasia. Several possible theories have been advanced to support the role of stenting in limiting intimal hyperplasia (32,63,64). However, there is minimal experimental evidence to justify this position, and available animal and clinical studies confirm that significant hyperplasia occurs within the stented segment. The extent and characteristics of this hyperplasia are illustrated by the case of a 67 year old man who had recurrence of angina 3 months after stenting in a bypass graft. Angiography revealed a severe narrowing within the stent that was treated by combined balloon angioplasty and atherectomy (Fig. 8). The tissue specimen removed by the atherectomy device is shown in Figure 9. The microscopic evaluation shows abundant extracellular collagenous matrix and areas of marked cellularity that stain positively for two smooth muscle cell cytoskeleton proteins, alpha actin and vimentin (Fig. 10).

Although hyperplasia is a consequence of stenting, the functional significance of this growth may be diminished. This is explained by the intrinsic dilating property of the self-expanding stent, which initially improves luminal area 50% more than angioplasty by itself and in many patients more than compensates for the late proliferation. Unfortunately, the ideal ratio of stent size to vessel size that will result in optimal dilation with minimal compensatory hyperplasia remains unknown. The importance of this relation to the final outcome was illustrated recently by a Mayo Clinic study (65) in which a model for restenosis was developed by implanting stainless steel and tantalum coils with markedly oversized angioplasty balloons inflated to high pressures up to 14 atm.

Confounding Aspects of Stenting

There are three additional aspects of stenting that further confound our understanding of the processes occurring in the vessel wall after injury.

1. Foreign body interactions with the vessel wall. In contrast to brief, transient balloon-induced injury, nonbiodegradable stents are permanent foreign bodies with potentially important interactions due to type of metal, electrostatic charges, and possible physical irritation from individual filaments. Whether the continued presence of a



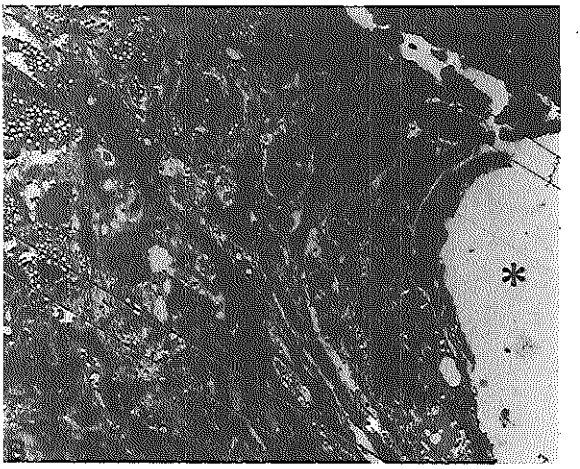
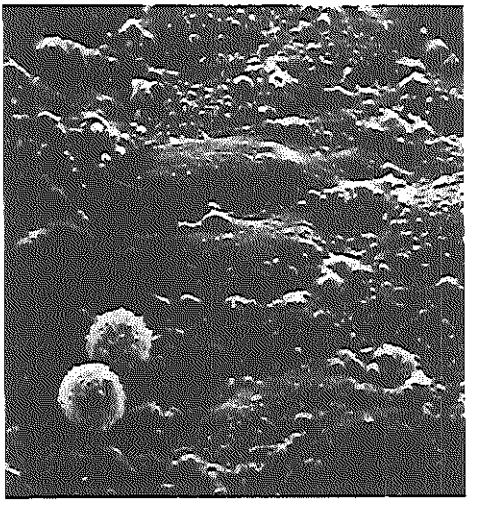


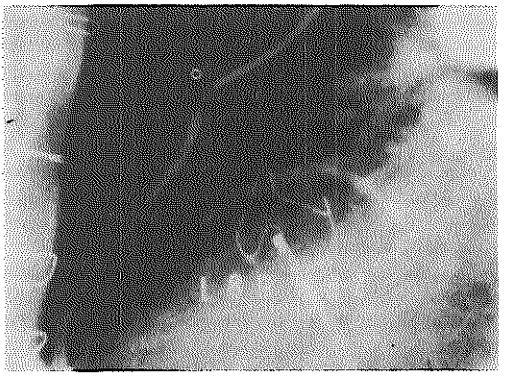
Figure 6. A. Transmission electron micrograph of saphenous vein bypass graft 6 months after stent implantation. Extracellular lipid deposits and cholesterol clefts (arrow) are evident alongside the foam cells. B. Higher magnification of another part of the section in A. An abundant number of foam cells can be seen. The diagonal lines are artifacts produced by the ultramicrotome. (*) = void representing the site of a removed 70 μm diameter stent wire. Abbreviations as in Figure 5.

Figure 7. Scanning electron micrograph of the endothelial lining shows an irregular, raised endothelial surface. Two leukocytes are adherent to the endothelium (bar = 5 μm).



foreign body in the vascular wall will continue to stimulate fibrointimal hyperplasia after the 6 months usually associated with balloon injury is also unknown. Concern has also been expressed as to whether the stent can trigger an allergic response, particularly in individuals who are hypersensitive to the individual metals that make up the device. Although there have been reports of transient inflammatory infiltrates in the adventitia after stent implantation, it is reassuring that there have been no reports of foreign body cells in the immediate vicinity of the implanted device in the experimen-

Figure 8. Coronary angiogram from a 67 year old patient who underwent stent implantation for a severe narrowing in the shaft of a 10 year old saphenous vein bypass graft. Three months later he was treated with atherectomy for restenosis within the stent.



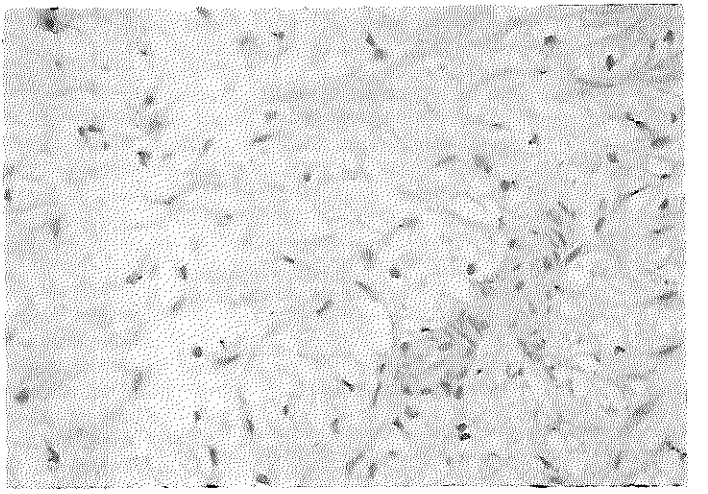


Figure 9. Light micrograph of hyperplastic tissue removed by atherectomy from the narrowed stent in Fig. 8. The specimen contains abundant smooth muscle cells and loose extracellular matrix. (Hematoxylin-eosin stain.)

tal animal model (14,15). This has been confirmed in limited experience in retrieved human stented coronary bypass grafts. In vitro attempts (Van der Giessen et al., unpublished observations) to identify endothelial membrane lipid peroxidation by free radicals formed in the presence of metallic stent elements have been unsuccessful, although the theoretic possibility exists.

2. Long-term effects of continuous barotrauma: role of exerted radial pressure. The chronic effects of continuous barotrauma induced by the expanded stent may have important ramifications. Because the Wallstent's properties are analogous to those of any spring, it tries to assume its equilibrium configuration, defined as the unconstrained diameter where net radial force is zero. If it is stretched beyond or constricted below this equilibrium, it generates forces to return to this configuration. We have studied the in vitro force-length relationship of the Wallstent stent (Fig. 11). These measurements have yielded calculations of the radial pressures exerted by this stent, both globally and locally at the site of the individual filaments if the stent is

Figure 10. Smooth muscle cells are identified in the restenosis tissue obtained at atherectomy by the dark brown staining. An antibody specific for smooth muscle alpha actin has been coupled to a peroxidase reaction and is responsible for the dark color.

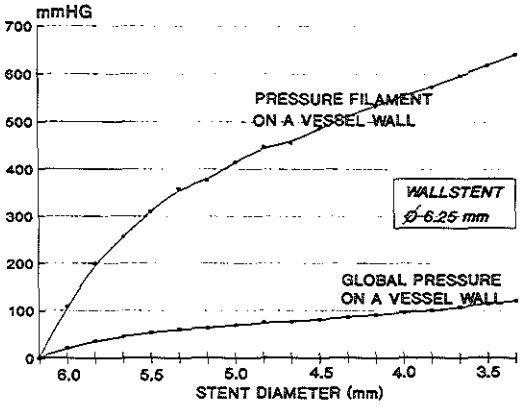


Figure 11. Radial pressures of the individual stent filament and the global pressure on the vessel wall of a self-expanding Wallstent (unconstrained diameter 6.25 mm) at varying degrees of expansion.

maintained at a diameter less than that of the unconstrained diameter. Significant pressures are generated by the stent (as in any spring-loaded device) to return it to its unconstrained size. For example, an unconstrained 6.25 mm diameter stent generates a radial global pressure of 50 mm Hg and a radial local pressure of about 300 mm Hg at the stent filament if it is maintained at 5.5 mm. This increases to 90 mm globally and 500 mm locally at 4.5 mm diameter. These pressure calculations would be additive to the mean arterial pressure and could have an important impact in situations where oversized Wallstents are implanted. In fact, localized areas of necrosis adjacent to the stent wires have been seen, which are probably the result of a pressure phenomenon.

3. Splinting the artery externally (casting) versus internally (stenting): effect on wall stress. Parallels have been drawn between the effects of splinting the artery externally (casting) and internally (stenting). Thubikar et al. (66) showed that externally casting segments of rabbit aortas limits pulsatile flow and atheroma development despite a high cholesterol diet. On the basis of this observation, others (32) have speculated that nonflexing internal stents may also reduce wall stress and consequently diminish hyperplasia formation. However, this analogy is not impressive. Although some of the pulsatile stretch may be borne by the external cast or the internal stent, and thus favorably affect wall stress, this is achieved by separate means. In the casting model, there should be a reduction in intramural wall stress, since the vessel is casted at a radius smaller than the maximal systolic expansion. In contrast to casting, stenting results in dilation of the artery and an increase in wall stress. This important stimulus to intimal hyperplasia appears to overcome the inhibitory effects of reduction in phasic vessel wall expansion. Booth et al. (67) modified the external casting model with interesting results. By applying an external nonoccluding Silastic collar on a rabbit carotid artery that did not affect end-systolic dimensions, they demonstrated that focal hyperplastic lesions rapidly develop. This finding supports the concept that external casting must decrease the vessel radius in order to achieve inhibition of intimal hyperplasia.

The State of Interventional Cardiology in 1990: Debulking Versus Dilating

Interventional cardiology has moved in two directions: devices that primarily dilate coronary narrowings (balloon angioplasty and stenting) and devices that physically debulk coronary tissue by extraction, liquefaction or vaporization (laser, directional and rotational atherectomy and spark erosion). At present, it is difficult to make comparisons among the various devices. However, there are several fundamental differences that may be important and merit further discussion.

Comparison of the various techniques. The ideal coronary intervention should selectively reduce the effect of the atheromatous lesion with minimal alteration of the normal vessel wall components and architecture. None of the currently available techniques completely satisfy these requirements. Balloon angioplasty, atherectomy (rotational and directional) and laser devices all cause extensive traumatic changes within the plaque and usually major alterations to the vessel wall architecture as well. Balloon angioplasty, the earliest intervention, has been shown to create tears and dissections within and at the edges of atherosclerotic plaques and frequently disrupts the internal elastic membrane and medial layers (68–71). Theoretically, these disruptions may be advantageous since the liberation of lipid and debris from the atheromatous lesions, a sort of debridement, may favorably affect the long-term biologic growth and behavior (if distal embolization of this material does not cause immediate clinical consequences) (72–74).

However, more important considerations may be the manner in which the healing process ensues in a damaged vessel with frayed, ragged membrane edges and separated muscular layers and the inherent problems of restoring the normal three-layered architecture of the arterial wall in an orderly fashion after such injury. Moreover, the extent of arterial disruption from angioplasty appears to be much less than in the actual removal of coronary tissue by debulking devices. Directional atherectomy in particular has been shown to be extremely effective in removing the atheroma but specimens include adventitia in up to 30% of cases (75), although there is no evidence to date that the rate of restenosis is related to the depth of the vessel wall extracted. Alternatively, stenting seems to be the least disruptive to the underlying architecture, although the underlying atheromatous lesion persists in the stented vessel with unknown future consequences. Stenting is able to "tack back" the cracks and tears induced by balloon angioplasty, which may

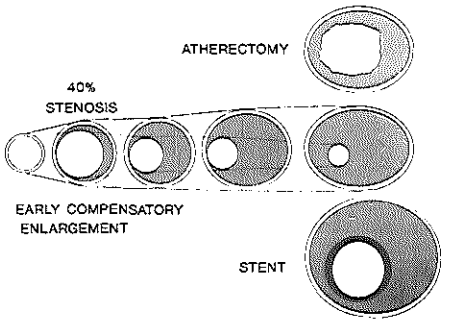


Figure 12. The natural progression of coronary artery disease as suggested by Glagov et al. (76) is illustrated in the **center row** by the early compensatory enlargement and the late luminal narrowing of progressive atherosclerosis. The differential effects of stenting and atherectomy in restoring the vessel diameter are shown. Stenting (**below**) restores the early compensatory enlargement in the vessel while it maintains the basic architecture of the vessel wall. By extracting vascular tissue, atherectomy (**above**) disrupts the underlying architecture of the wall. Modified from Glagov S et al. (76) with permission.

diminish the stimulus for fibrosis in much the same way that a properly closed wound minimizes fibrotic scar in the healing phase.

Glagov revisited. Glagov et al. (76) observed that the diseased coronary artery is able to adapt to progressive plaque expansion by enlarging the size of the vessel. This compensatory mechanism maintains the luminal area until the plaque lesion occupies 40% of the area inside the internal elastic lamina, beyond which progressive luminal narrowing occurs. In other words, significant atherosclerosis can coexist with normal or even enlarged luminal area until the limits of this adaptation are exceeded.

Striking similarities exist between the chronic process of atherosclerosis and the situation in the stented vessel wall. The stent is initially embedded in the intima, which results immediately in enlargement of the lumen and later in localized medial thinning at the site of the stent wires, a commonly observed pathologic feature of atherosclerosis. Stenting may be regarded as the invasive cardiologist's attempt to restore the aforementioned "Glagovian" balance between plaque and luminal area, but in vessels that contain plaques >40% of the internal elastic lamina (Fig. 12). Stents effectively alter the relation between plaque size and lumen area, resulting in a shift in the curve. Progressive vessel dilation by the stent can maintain adequate luminal area unless excessive fibrointimal hyperplasia upsets the new balance.

Expansion ratio. Expansion ratio is an important concept that relates the final effect on the arterial diameter to the size of the catheter required to deliver this effect (32) (Table 1). A

Table 1. Expansion Ratios With the Coronary Interventional Devices

Intervention	Procedures	Device Profile (mm)	Vessel Diameter Preprocedure (mm)	Maximal Achievable Diameter/(Range) (mm)	Postprocedure Diameter (mm)	Theoretic Expansion Ratio	Effective Expansion Ratio
Balloon angioplasty	443	0.7–1.3	1.1 ± 0.3	2.9 ± 0.4 (2.0–3.5)	1.8 ± 0.4	2.2–4.1	1.4–2.6
Stenting							
Self-expandable	357	1.6	1.3 ± 0.7	4.0 ± 0.7 (2.5–6.0)	2.6 ± 0.6	2.5 (1.6–3.8)	1.6
Balloon-expandable	27	1.4–1.6	1.0 ± 0.3	3.3 ± 0.3 (3.0–4.0)	2.4 ± 0.3	2.1–2.4	1.5–1.7
Atherectomy							
Directional	39	2.1–2.5	1.1 ± 0.4	3.3 ± 0.5* 2.0 ± 0.2†	2.5 ± 0.6	1.3–1.6	1.0–1.2
Rotational	52	1.5–2.0	0.9 ± 0.3	1.9 ± 0.3 (1.5–2.0)	1.7 ± 0.4	1.0	0.9–1.1
Excimer laser	55	1.4	0.5 ± 0.4	1.4	1.7 ± 0.5	1.0	1.2

*With balloon inflated; †with balloon deflated. This table compares the device profile and immediate angiographic results of several interventions. The profile of the device is based on data on 2.0 to 3.5 mm diameter balloon catheters (77), the Wallstent (Medinvent) self-expandable stent, Wiktor (Medtronic) balloon-expandable stent, Simpson Coronary Atherocath (DVI) directional atherectomy device, Rotablator (Heart Technology) rotational atherectomy device and the model Max-10 excimer laser (Technolas, Munich). The relation between the profile of the device and the maximal achievable diameter of the device is the theoretical expansion ratio. The maximal achievable diameter of the vessel is calculated according to the size of the device while it is operational in the coronary vessel. In the case of balloon angioplasty, balloon-expandable stent and directional atherectomy, the maximal achievable diameter corresponds to the diameter of the device while the balloon is inflated and to the unconstrained diameter of the self-expandable stent. The rotational atherectomy device and the excimer laser do not alter their diameter during the procedure. The postprocedure diameter is measured immediately after the procedure. The effective expansion ratio represents the ratio between the postprocedure result and the profile of the device and thus indicates not only the initial effect of the device but also the effect of elastic recoil, which is primarily responsible for the deterioration in the diameter from the maximal achievable diameter to the postprocedure diameter. The diameter values listed are the mean value ± SD of the different-sized devices from each interventional study; ranges are in parentheses. The preprocedure data, which may also affect the postprocedure result, were similar for all interventions (0.9 to 1.3 mm) except for the excimer laser, which may explain a somewhat lower postrecoil diameter and effective expansion ratio. The quantitative angiographic data for all devices except the rotational atherectomy device (Peterson K, unpublished observations) and the excimer laser (79), were collected at the Thoraxcenter.

favorable ratio is best exemplified by a small catheter delivery system that is able to pass severely narrowed segments and yet optimally dilate the stenosis. The maximal effect of the device may be partially lost because of the elastic recoil of the vessel. The current interventional devices may have differential effects in these two areas: the immediate result when the device is initially used, and then the partial loss of the initial gain after the device has been removed. An attempt has been made to separate these two effects by subdividing the expansion ratio into the theoretical expansion ratio (a measure of the effect while the device is operational) and the functional expansion ratio (which takes into account the elastic recoil phenomenon). For example, a 4 mm diameter balloon angioplasty catheter should achieve a vessel diameter of 4 mm at the time of balloon inflation but this is reduced immediately after deflation, primarily because of the elastic recoil of the vessel. Balloon angioplasty and stenting give extremely favorable theoretic and effective expansion ratios since they may be delivered on low profile catheters. The wide range for the theoretic and effective expansion ratios seen with balloon angioplasty is explained by the variation in the size of the balloons (2.0 to 3.5 mm) used in the study from which these data were obtained. The atherectomy devices are more limited by the profile of the device that is introduced into the coronary artery. The dimensions of the rotational atherectomy device and the excimer laser do not change while in operation and therefore both exhibit lower theoretic expansion ratios. However, by physically removing or vaporizing tissue, the potential elastic recoil effect is diminished by atherectomy and excimer laser devices.

Conclusion

Once again the question is asked: Can the promises of the new technology, and in particular the coronary stent, ever be realized?. Stenting will only achieve clinical acceptance when the safety, efficacy and cost efficiency are superior to those of balloon angioplasty alone. Safety remains the major limitation of stenting. In the initial 105 patients with an implanted Wallstent, 20% had documented occlusion within the 1st 14 days, usually resulting in myocardial infarction and in some cases necessitating emergency bypass surgery. With further experience this was reduced to 13% in the next 100 patients. Schatz et al. (15) recently reported a 3.6% subacute occlusion rate in contrast to a 16% rate in their early experience when warfarin treatment was omitted (78). However, the price of chronic anticoagulation therapy—bleeding complications, prolonged hospitalization to initiate therapy and effects on the quality of life—must also be considered. With the increasing importance of third party payment, the cost differential among competing therapies will also dictate medical policies. Finally, these devices must show beneficial effect on late restenosis in order to gain clinical acceptance. The late follow-up results of quantitative coronary angiography for two of the stents will be published in the next several months and should provide more objective evidence with which to evaluate the future role of the stent.

The early experience of modern interventional cardiology evokes lessons contained within Pandora's box. In this classic Greek myth, man, beguiled by the attraction of Pandora opened the box that exposed heretofore unknown perils and disease, while Hope (Elpis), the lone content of the box that could be controlled by man, remained hidden deep within the box. The allure of the newer techniques and devices in interventional cardiology, like Pandora herself, have brought us a new set of problems that we have been ineffectual in solving. However, Hope also exists within the modern Pandora's box and our capacity to realize hope will depend on a scientific approach to the problems of restenosis and neointimal hyperplasia in a mutual effort (in concert with industry) of interventional cardiologists, pathologists, molecular biologists, biochemists, pharmacologists and our patients, the general public.

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Chapter 8

Enhanced angiotensin II degradation in porcine coronary neointimal hyperplasia induced by stent implantation

LABORATORY INVESTIGATION

Enhanced angiotensin II degradation in porcine coronary neointimal hyperplasia induced by stent implantation

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Background: Angiotensin II (Ang II) has been proposed as a modulator of growth factor responses in the arterial wall. Employing a model of stent-induced neointimal hyperplasia, we studied angiotensin I (Ang I) elimination.
Methods: Balloon-expandable radiopaque stents (n=6) were implanted in coronary arteries of pigs. After 3 months, the stented and nonstented (control) vessels were studied *in vitro* for their conversion of radiolabeled ^{125}I-Ang I to ^{125}I-Ang II in the presence or absence of captopril. Conversion was also studied after removal of the endothelium.
Results: Immunocytochemistry confirmed the presence of endothelium covering the neointima. Stented vessels metabolized ^{125}I-Ang I faster and released less ^{125}I-Ang II than normal arteries. ^{125}I-Ang I formation could be completely blocked by captopril, but only up to 75% by removal of the endothelium. Determination of the rate constants for elimination of ^{125}I-Ang I revealed that the reduced release of ^{125}I-Ang II appeared not to be due to decreased conversion by angiotensin-converting enzyme in stented vessels, but merely to increased degradation.
Conclusions: Porcine coronary arteries up to 3 months after stent implantation release significantly less ^{125}I-Ang II upon challenge with ^{125}I-Ang I. A higher degradation of ^{125}I-Ang II in the stented coronary arterial wall may explain this finding. Enhanced degradation of pro-, but likely also of antiproliferative, peptide growth factors locally in the vessel wall may further complicate our understanding of neointimal proliferation after arterial damage.

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Keywords: angiotensin converting enzyme, endothelium, pig, restenosis, stents

The recurrence of coronary artery stenosis at the site of earlier therapeutic intervention is a major limitation of percutaneous transluminal coronary angioplasty (PTCA) [1–3]. Histopathologic studies have identified fibrocellular neointimal hyperplasia as the main morphologic feature of this restenosis process [4–7]. Shimokawa *et al.* [8,9] in porcine coronary arteries and Weidinger *et al.* [10] in rabbits have shown that regeneration of endothelium covering this neointimal hyperplasia occurs, but that these vessels fail to produce endothelium-derived relaxant factor (EDRF) during the first weeks after experimental PTCA. This period of endothelial dysfunction coincides with the fastest growth rate of neointimal build-up. Regeneration of an endothelial lining has also been demonstrated early af-

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ter implantation of stents in normal porcine coronary arteries [11]. Despite this endothelial covering, the thickness of the neointimal layer continues to increase up to 4 weeks after implantation.

Angiotensin II (Ang II) has been proposed as a modulator of growth factor responses in the arterial wall [12], but can also regulate gene expression itself [13], and is locally synthesized by endothelial cell-associated angiotensin-converting enzyme (ACE) [14], although some ACE activity may also be present in other layers of the vessel wall [15]. Inhibition of ACE by cilazapril and captopril has been reported to reduce experimental balloon damage-induced hyperplasia [16]. Therefore, we tested the hypothesis that the regenerated endothelium may not only be dysfunctional in its EDRF release, but also that the local angiotensin metabolism is abnormal. The study was performed in a pig model of stent-induced neointimal hyperplasia.

Methods

Balloon-expandable intracoronary stent

The balloon-expandable stent used in this study (Wiktor, Medtronic Inc., Minneapolis, MN, USA) is constructed of a single tantalum wire (0.127 mm in diameter) [11] that is formed into a sinusoidal wave and wrapped into a helical coil structure. The features of this prosthesis design are such that by inflation of the balloon, the diameter of the stent increases without alteration of its length. The maximal diameter of the balloon after inflation determines the ultimate size of the prosthesis after implantation. The balloon diameters of the mounted angioplasty catheters used in this study were 3.0 and 3.5 mm, and the lengths of the prostheses 14 to 16 mm.

Animal preparation

Experiments were performed in Yorkshire pigs (39 to 41 kg; HVC, Hedel, the Netherlands). The investigations were performed according to the *Guide for the Care and Use of Laboratory Animals* [17], and the protocol was approved by the Committee on Experimental Animals of Erasmus University. After an overnight fast, the animals were sedated with 20 mg/kg ketamine hydrochloride. After endotracheal intubation, the pigs were connected to a ventilator that administered a mixture of oxygen and nitrous oxide (1:2, vol/vol). Anesthesia was maintained with 1 to 4 vol% enflurane, while pancuronium bromide was used as a muscle relaxant. Antibiotic prophylaxis was administered by an intramuscular injection of 1000 mg of a mixture of procaine penicillin-G and benzathine penicillin-G.

Under sterile conditions, an arteriotomy of the left carotid artery was performed and a 9F introduction sheath was placed. Then 5000 IU heparin sodium was administered and an 8F guiding catheter was advanced to the ascending aorta. After measurement of arterial blood pressure and heart rate, and withdrawal of an arterial blood sample for the measurement of blood gases and acid-base balance, left and right coronary angiography was performed using iopamidol (Iopamiro 370, Dagra BV, Diemen, the Netherlands) as contrast agent. Three animals underwent the catheterization procedures.

Stent implantation

From the angiograms, and using the diameter of the guiding catheter as a reference, a segment with a diameter of 2.5 or 3.0 mm was selected in two of the three large coronary arteries (left anterior descending coronary artery [LADCA], left circumflex coronary artery [LCXCA], right coronary artery [RCA]). No attempt was made to avoid side branches or angulated coronary segments. Thereafter, a 3.0-mm (for 2.5-mm coronary segments) or a 3.5-mm (for 3.0-mm coronary segments) balloon angioplasty catheter with a stent coil crimped on its deflated balloon was advanced over a 0.014-inch steerable guidewire to the site preselected for implantation. After administration of additional 2500 IU heparin through the guiding catheter, the balloon-expandable stents were placed as described in detail [11]. After repeat angiography of the stented coronary arteries, the guiding catheter and the introducer sheath were removed, the arteriotomy repaired, and the skin closed in two layers. The animals were allowed to recover from anesthesia. Antithrombotic drugs were not administered after the procedure.

Follow-up angiography

The catheterization procedure for follow-up angiography at 12 weeks was identical as described above. Coronary angiograms were recorded using the same projection and identical geometry of the x-ray equipment as during implantation. Thereafter, the thorax was opened by a midsternal split, a lethal dose of sodium pentobarbital injected intravenously, immediately followed by cross-clamping of the ascending aorta. After puncturing the aortic root above the coronary ostia, 500 mL of saline was infused under a pressure of 120 mm Hg. Then the heart was excised and the coronary arteries dissected from the epicardial surface. The stented segments and adjacent unstented segments were divided in equal parts. One part was placed in 4% paraformaldehyde in phosphate buffer (pH 7.3) for at least 48 hours in preparation for microscopy, whereas the other half was placed in Krebs-Henseleit buffer at 37°C for *in vitro* measurements of angiotensin metabolism.

Quantitative angiographic analysis

Coronary angiograms (preimplantation, immediately after implantation, and after 12 weeks) were analyzed using the quantitative coronary angiography analysis system [18]. Mean luminal diameter at the site of stent placement was measured.

Microscopic examination

After fixation, the specimen were processed for light microscopical examination, as described earlier [11]. Qualitative examination of the neointimal layer and the presence of endothelium was performed after staining with hematoxylin-azophloxin and the elastin stain resorcin-

fuchsine, using a light microscope (BH2, Olympus, Tokyo, Japan).

Identification of endothelium

The horseradish-labeled lectin *Bandeiraea simplicifolia* (BSI-B4, Sigma Chemical Company, St. Louis, MO, USA) was used as an endothelial marker [19]. After rehydration, the sections were exposed to normal rabbit serum (10% in phosphate buffered saline) for 15 minutes at 37°C, followed by BSI-B4 (3 to 4 μg/mL) for 30 minutes at 37°C. The peroxidase reagent diamino benzidine (0.67 mg/mL, Sigma Chemical Company, St. Louis, MO, USA) was used as a detecting reagent.

Conversion of ^{125}I-angiotensin I by isolated coronary arteries

One half of each vessel was used for the study of production of ^{125}I-Ang II from ^{125}I-Ang I. The vessels were dissected free from surrounding tissue and cut helically. The resulting strips were then divided in two equal pieces. Both pieces were incubated *in vitro* at 37°C in 6 mL of Krebs-Henseleit solution. Prior to and during the experiment, the solution was oxygenated using a mixture of 95% O_2 and 5% CO_2. In three additional normal vessels, the endothelium was removed by inserting the tips of a watchmaker's forceps into the lumen and rolling the vessel back and forth over a saline-loaded filter paper, before cutting the vessel helically [20]. The removal of the endothelium was checked histologically.

The experiments were started by adding at $t=0$ minutes approximately 10^5 counts per minute (cpm) of purified mono-iodinated ^{125}I-Ang I (specific activity, 3.6 $\times 10^6$ cpm/pmol [21]). Samples of 200 μL were taken at 0, 5, 10, 20, and 30 minutes, and immediately mixed with 25 μL of inhibitor solution that contained 125 mM disodium EDTA and 25 mM 1,10-phenantroline, to inhibit ACE and angiotensinases, respectively. The samples were kept on ice and extracted by reversible adsorption to octadecylsilyl silica (SepPak C18, Waters, Millford, MA, USA) as described before [21]. ^{125}I-Ang I was separated from its metabolites by reversed phase high-pressure liquid chromatography (Fig. 1) [21]. After separation by high-pressure liquid chromatography, the concentrations of ^{125}I-Ang I and its metabolites were measured in the gamma counter. Results are expressed as a percentage of total cpm recovered after SepPak extraction, corrected for either protein content or wet-tissue weight. Wet-tissue weight was obtained by weighing the tissues after blotting on dry paper.

Protein analysis

Pieces of arteries that were incubated for *in vitro* ^{125}I-Ang I metabolism were homogenized with a polytron PT10. The homogenate was then dissolved overnight in 1M NaOH at 35°C. Protein was determined by the biuret reaction, using serum albumin as a standard [22].

Statistical analysis

All data have been expressed as mean±SEM, unless otherwise stated. The significance of the changes in the data were evaluated by analysis of variance. A P value less than 0.05 was considered statistically significant.

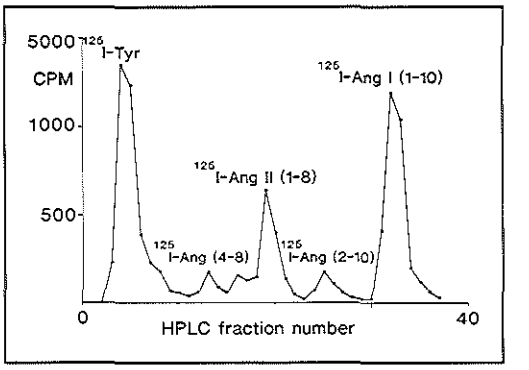

Fig. 1. High-pressure liquid chromatography (HPLC) elution profile of ^{125}I-labeled angiotensins. The numbers in parentheses denote the *N*-terminal amino acid sequences released by isolated coronary artery segments upon challenge with ^{125}I-Ang I.

Results

Systemic hemodynamics and blood gases during implantation and follow-up angiography

During implantation and follow-up angiography, heart rate (123±24 bpm and 102±25 bpm, respectively), systolic arterial pressure (130±16 and 112±5 mm Hg) and diastolic arterial pressure (105±18 mm Hg and 91±13 mm Hg) were comparable. The oxygenation of arterial blood and acid-base balance were also similar during stent-placement and follow-up angiography and within the normal range (pH, 7.38±0.05; pO_2, 157±34 mm Hg; pCO_2, 46±6 mm Hg).

Placement of stent and follow-up angiography

A total number of six stents were evenly distributed over the coronary arteries: LADCA (one 3.0-mm-diameter stent, one 3.5-mm stent), LCXCA (two 3.5-mm stents), and RCA (two 3.5-mm stents). Quantitative analysis of the angiograms showed that the mean coronary diameter was 3.1±0.2 mm before stent placement, 3.2±0.1 mm immediately after placement, and 3.2±0.2 mm after 3 months. Mean balloon diameter during implantation was 3.4±0.1 mm, which means that the vessels were overstretched by 9%±5% acutely ($P<0.05$) and that elastic recoil of the stented segment was 5%±2% (P is not significant).

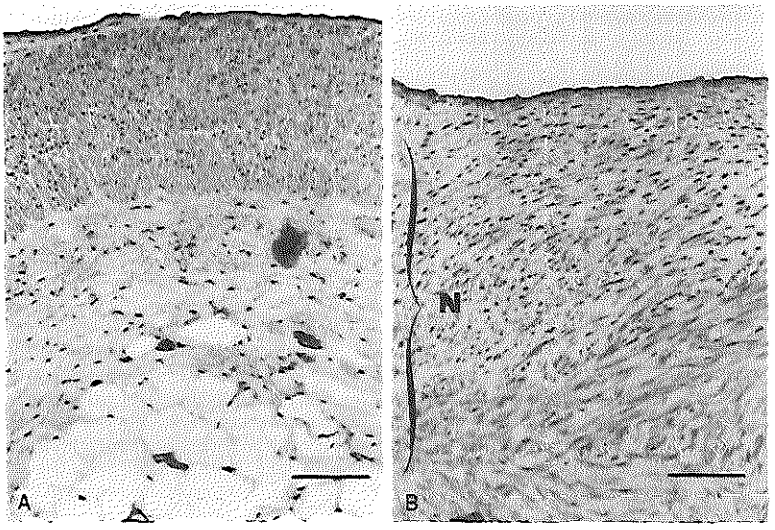

Fig. 2. A, Light microscopy of a control coronary artery (left circumflex coronary artery) showing a positive stain for the endothelial marker *Bandeiraea simplicifolia*. Please note that as a positive internal control, the capillary endothelium in the adventitia is also stained. *Bar* represents 100 μm. **B**, Light microscopy of the left anterior descending coronary artery 12 weeks after stenting. The neointima (N) is covered by a continuous layer of endothelium, as confirmed by positive staining for the lectin *Bandeiraea simplicifolia*. *Bar* represents 100 μm.

Histology

Morphology of stented and control vessels

The stented coronary arteries all showed a cellular, layered neointima of limited thickness on top of and in between the stent wires. Most of the intimal cells were smooth muscle cells with their typical organization in a collagenous extracellular matrix. Specifically, around the wires, but to a lesser extent also in the subendothelial space, white blood cells were found. The stent mildly compressed the arterial media. In the control vessels, this intimal thickening with smooth muscle cells and white blood cells was not observed.

Identification of endothelium

Immunocytochemical analysis of four stented coronary segments and two control (nonstented) coronary segments showed a positive staining for the lectin *Bandeiraea simplicifolia* in all cases of the cell layer lining the lumen of the vessel (Fig. 2). The absence of endothelium in the denuded control arteries was also confirmed histologically and by scanning electron microscopy.

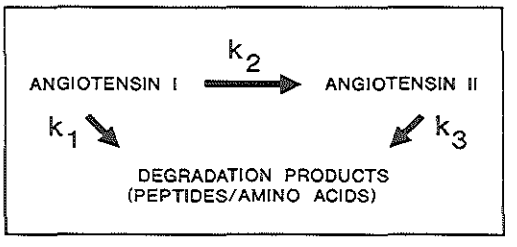

Fig. 3. Scheme of elimination of angiotensin I and angiotensin II. k_1 and k_2 are the elimination rate constants for degradation and conversion of angiotensin I, respectively. k_3 is the rate constant of degradation for angiotensin II and identical to k_1 [47].

Conversion of ^{125}I-angiotensin I by isolated coronary arteries

Isolated coronary arteries rapidly metabolized ^{125}I-Ang I. SepPak extraction and high-pressure liquid chromatography separation showed that various radiolabeled metabolites appeared in the medium upon challenge with ^{125}I-Ang I. Peaks with retention times corresponding to those of ^{125}I-tyrosine, ^{125}I-Ang-(pentapeptide 4-8), ^{125}I-Ang II, and ^{125}I-Ang-(nonapeptide 2-10) could be readily identified (Fig. 1). Not unexpectedly, most radioactivity concentrated in the ^{125}I-tyrosine peak at the end of the experiment. Virtually no radioactivity could be detected in the tissue after the experiment, suggesting that ^{125}I-Ang I metabolism occurred predominantly extracellularly.

^{125}I-Ang I is eliminated either by conversion to ^{125}I-Ang II by ACE or by degradation to small, biologically inactive peptides by other enzymes (Fig. 3). The elimination rate constants of ^{125}I-Ang I in the various coronary vessels have been presented in Table 1. All elimination rate constants were calculated as described in Appendix 1. The overall elimination rate constant k_{el} was higher in the stented coronary arteries than in the normal coronary arteries. No differences were found for k_{el} between de-endothelialized and normal coronary vessels.

The amount of ^{125}I-Ang II found in the medium during incubation with ^{125}I-Ang I is shown in Figures 4 and 5. Stented coronary arteries released approximately 50% less ^{125}I-Ang II than normal coronary

Table 1. Elimination rate constants of ^{125}I-angiotensin I in the control, stented, and deendothelialized coronary arteries*

Arteries	k_{el}	k_1	k_2
Control	1.42±0.01	0.93±0.14	0.49±0.12
Stent	1.99±0.18	1.38±0.19	0.61±0.01
No endothelium	1.45±0.21	1.31±0.20	0.14±0.03

*Values (mean ±SEM) have been expressed per gram wet weight.
k_{el}—overall elimination rate constant; k_1—rate constant for degradation; k_2—rate constant for conversion by angiotensin-converting enzyme.

arteries upon challenge with ^{125}I-Ang I (Fig. 4). After removal of the endothelium of the normal, nonstented vessels, the release of ^{125}I-Ang II was reduced to 20% to 30% of control (Fig. 5). Following ACE inhibition, the amount of radioactivity in the ^{125}I-Ang II peak was reduced to background values, showing that ACE is the main, if not the only, enzyme responsible for ^{125}I-Ang II formation in isolated coronary arteries. The elimination rate constant of ^{125}I-Ang I measured in the presence of captopril (k_1) was smaller than the elimination rate constant measured in the absence of captopril (k_{el}). By subtracting k_1 from k_{el}, we calculated that the elimination rate constant for conversion (k_2) was similar in stented and normal, nonstented vessels. Therefore, it follows that the decreased release of ^{125}I-Ang II from stented vessels does not appear to be due to a lesser degree of conversion, but was merely the consequence of an increased degree of degradation (larger k_1). In de-endothelialized vessels, k_2 was approximately 75% lower than in the other two types of vessels. This shows that approximately 25% of the ACE activity in coronary arteries is not due to endothelial-bound ACE.

Discussion

Experimental model of neointimal proliferation

Pharmacologic prevention of restenosis after PTCA has thusfar been proven to be rather disappointing [23–25]. Positive [16,26,27] as well as negative [28–30] results employing ACE inhibition in animal models have been reported. Although "no single model of vessel wall injury has yet been shown to reliably predict the impact of pharmacologic intervention on the incidence of restenosis in humans" [31], these animal studies have considerably stimulated the interest in the (patho)physiologic role of the local coronary renin-angiotensin system.

Overstretching of the coronary arterial wall by stents has been shown both experimentally [32] and clinically [33] to lead to an excessive proliferative response within the stent. Quantitative angiography showed that the stents in the present study did not overstretch the vessel wall, and the extent of neointimal hyperplasia in our experiments was, therefore, limited. Proof that we are dealing with neointimal hyperplasia and not with recanalized thrombus in the current study may be formed by the typical histologic features of the stented coronary segments [11]. In addition, the absence of left ventricular scarring in the distribution of the stented coronary arteries in this species without coronary collateral vessels, where a temporary occlusion of only 60 minutes duration causes irreversible myocardial damage [34], is another argument against thrombus recanalization. Further indirect evidence against a thrombotic origin of the neointima is the fact that all animals survived the implantation and follow-up period, whereas a high incidence of lethal arrhythmias occurs in pigs after only brief periods of abrupt coronary artery occlusion [35], as well as in a model of more gradually developing coronary thrombosis [36]. The lining cells of this neointima were identified as endothelium. Nevertheless, we were able to observe pronounced differences in the release of ^{125}I-Ang II as compared with those of normal, nonstented vessels.

Elimination of angiotensin I by conversion and degradation

Angiotensin I is eliminated by both conversion to Ang II (elimination rate constant k_2) by ACE and by degradation to smaller inactive fragments (elimination rate constant k_1). Ang II is eliminated by degradation only (elimination rate constant k_3) (Fig. 3).

We found that k_2 was similar in stented and normal vessels. This suggests that approximately the same activity of ACE is present in both types of vessels. Captopril almost completely prevented the release of ^{125}I-Ang II by isolated vessels upon challenge with ^{125}I-Ang I (Figs. 4 and 5), proving that ACE is the most important, if not the only, enzyme responsible for the conversion of ^{125}I-Ang I to ^{125}I-Ang II in isolated coronary arteries.

Angiotensin-converting enzyme seems not to be limited to the endothelium, because ^{125}I-Ang II was also released from de-endothelialized vessels, although in much lower quantities (Fig. 5). Based on the elimination rate for conversion in de-endothelialized vessels, it could be estimated that approximately 25% of the total amount of ACE in normal coronary arteries was situated outside the endothelium (Table 1).

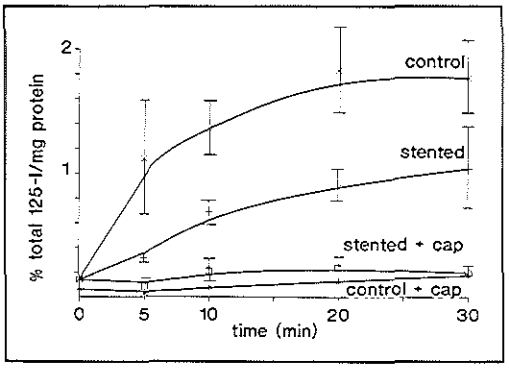

Fig. 4. Release of ^{125}I-Ang II during incubation with ^{125}I-Ang I. The stented segments (stented) released 50% less Ang II as compared with not stented (control) vessels. In both types of vessels, the Ang II release could be blocked virtually completely by captopril (cap). Results (mean±SEM) are expressed per milligram protein.

The fact that the elimination rate constant for conversion (k_2) was not different in stented and normal coronary vessels does not necessarily imply that this similar ACE activity in stented vessels was located at the newly formed endothelium. Rakugi *et al.* [37], for instance, recently reported in preliminary form an enhancement of ACE expression in the neointima after experimental balloon angioplasty. Repeating our measurements in stented arteries with removed endothelium may yield a more precise location of neointimal ACE activity.

Despite the fact that the elimination rate constant for conversion was not different in stented and normal, nonstented coronary vessels, stented vessels released approximately 50% less ^{125}I-Ang II into the medium upon challenge with ^{125}I-Ang I (Fig. 4). The overall elimination rate of ^{125}I-Ang I was higher in stented vessels than in normal vessels. This appeared to be due to a higher elimination rate constant for degradation (Table 1). A higher degradation rate in stented vessels would not only cause ^{125}I-Ang I to be eliminated faster, but ^{125}I-Ang II too. If ^{125}I-Ang II is more rapidly degraded in stented coronary arteries than in normal coronary arteries, this also would cause a reduction in the release of ^{125}I-Ang II by these vessels into the medium.

Possible mechanism of higher angiotensin degradation in neointimal hyperplasia

Several factors may be responsible for this higher degradation of angiotensins. First, it has been shown that experimental balloon angioplasty [38], and also the implantation of foreign bodies like stents in animals [39,40] as well as in humans [41], leads to an increased and persistent presence of white blood cells in the neointima. Although we did not measure the amount of white blood cells in the present protocol, it is possible that leukocyte-derived peptidases and proteases are partly responsible for the enhanced degradation of polypeptides such as angiotensin. Second, after stent implantation and probably also after other interventional techniques such as balloon angioplasty, the newly formed endothelium may have lost its barrier function and is consequently more permeable to the penetration of Ang I or Ang II, which is formed at the luminal side and is subsequently degraded by neointimal peptidases. This impaired barrier function could be caused by enhanced transendothelial vesicular transport or passive influx of molecules due to loose intercellular junctions [42,43]. In the same animal model but using another stent device, we were able to demonstrate such a decreased endothelial barrier function, using the Evans blue dye exclusion test [40].

Third, the k_1 values of Table 1 suggest that degradation of de-endothelialized normal vessels is similar to that of neointima. Therefore, degradation in normal vessels without endothelium also appears to be enhanced. This also may be explained by increased access of Ang II to subintimal vessel wall enzyme activity in the extracellular matrix. This shows that the normal media (either the smooth muscle cells or proteases present in the extracellular matrix) can be fully responsible for this rate of degradation. Next to the impaired or absent endothelial barrier in stented and de-endothelialized arteries, respectively, damage to the internal elastic lamina, which can act as a molecular sieve, also may be responsible for an increased access of molecules such as angiotensin to subintimal enzyme degradation [44].

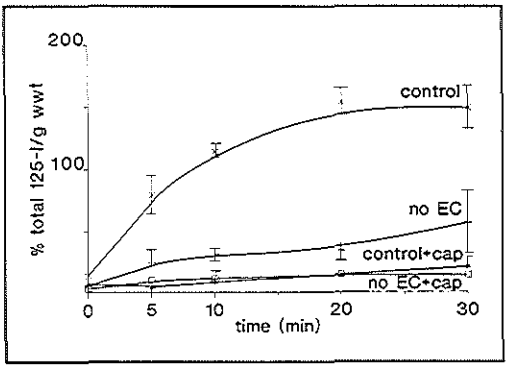

Fig. 5. Release of ^{125}I-Ang II during incubation with ^{125}I-Ang I in the presence (+cap) or absence of captopril. Results (mean ±SEM) are expressed per gram wet weight. No EC—without endothelium.

Conclusions

In conclusion, the present *in vitro* study indicates that ACE appears to be the main angiotensin II-forming enzyme in normal as well as stented porcine coronary arteries. In addition, ACE is not limited to the endothelium. Most prominently, in both de-endothelialized normal vessels and in endothelialized vessels containing mild neointimal hyperplasia, degradation of angiotensin I and II is enhanced, thereby indicating the activation of the local renin-angiotensin system 3 months after stenting. A teleologic explanation for this finding may be the necessity of a local mechanism that eliminates Ang I and Ang II more rapidly and thereby prevents vasoconstriction, smooth muscle cell growth, and collagen synthesis by this activated renin-angiotensin system.

This study contributes to the accumulating evidence that the regenerated vessel wall suffers a (temporary) modification or loss of important functions after a proposedly therapeutic intervention. It is, however, premature to extend the findings of the present study to human clinical restenosis after angioplasty [45]. Nevertheless, it is tempting to speculate that our result of similar ACE activity in arteries exhibiting neointimal hyperplasia compared with normal arteries provides a pathophysiologic basis for the failure of converting enzyme inhibitors (captopril, enalapril, and cilazapril) to prevent both the fibrocellular response to injury in pigs [28,29] and nonhuman primates [30], and the restenosis rate after coronary angioplasty in patients with coronary artery disease [46]. Moreover, if confirmed in *in vivo* animal models and humans, our finding of enhanced local degradation of pro-, but most likely also of antiproliferative peptide growth factors in the vessel wall, may further complicate our understanding of neointimal proliferation after arterial damage.

Appendix I: calculation of the elimination rate constants

^{125}I-Angiotensin I is eliminated by conversion to ^{125}I-Ang II by ACE or by degradation to small biologically inactive peptides by other enzymes. The respective first order rate constants are k_2 for conversion and k_1 for degradation. The overall elimination rate constant (k_{el}), measured in the absence of captopril, involves both conversion and degradation ($k_{el} = k_1 + k_2$). In the presence of captopril, the elimination of ^{125}I-Ang I involves degradation only ($k_{el} = k_1$). The difference between the elimination rate constants measured in the presence and absence of captopril equals the elimination rate constant for conversion ($k_{el} - k_1 = k_2$).

After measurement of the concentration of ^{125}I-Ang I at the start (C_0) and at a specific time point (C_t) during the experiments, the rate constants of overall elimination can be calculated from:

$$C_t = C_o \cdot e^{-k_{el} \cdot t}$$

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Chapter 9
General Discussion.

Aim of this Thesis.

Stents, when used as endovascular prostheses, give inner mechanical support to the vessel wall. As such, they will exert either passive (balloon-expandable stent) or active (self-expanding stent) outward pressure, which is required to remain positioned and maintain an appropriate lumen. The device will initially interact with blood and will become embedded in the vascular wall during the process of woundhealing, where it will interact with the vascular tissue.

The aim of this thesis was to increase our knowledge on the effect of stenting, to assess the acute damage to the vessel wall, thrombogenicity of the devices, and the short- and long- term effects of these implants on the vascular wall.

Acute Injury and the Effect of Stenting.

Implantation of stents is always accompanied by damage to the endothelial lining and stretching of the vessel wall. The extent of acute injury depends on several factors such as stent design, means of delivery, lesion morphology and additional balloon angioplasty[1].

Endothelium. The vein-graft study reported in Chapter 5 showed that with the balloon expandable Wiktor stent, the acutely visible damage is probably limited. Contrary to our expectations, the endothelial lining seemed intact except for the areas in direct contact with the wires. The same phenomenon was observed in human saphenous vein bypass grafts stented with the self-expanding Wallstent (Chapter 6). Scanning electron microscopy again revealed patches of seemingly undamaged endothelium between the wires.

The vascular wall. When implanting a stent, the wires selectively impress the underlying tissue. This yields a luminal appearance with a waveform geometry. Especially the vein grafts studied in Chapters 5 and 6 displayed this phenomenon. Due to the open sinusoidal configuration of the Wiktor stent, the parts of the vessel wall that were not covered by the wires, were bulging out into the lumen. This probably results in disturbed flow patterns which can augment thrombus deposition[2]. The tighter weave of the Wallstent prevents the extreme waveform geometry as observed with the Wiktor stent.

In the stented porcine arteries, the stent wires usually impress the media with 20-30% as compared to the adjacent "within stent" media, sometimes lacerating the Lamina Elastica Interna. At one week this pressure results in a slightly acellular media directly underneath the

stent wire. Other research groups report similar findings on endothelial and medial damage[3,4].

The observations regarding surface geometry and prosthesis-restricted initial damage, implicates that a higher prosthesis-covered to non-covered surface ratio yields a higher initial damage to the endothelium and probably the underlying tissue, but a smoother and perhaps less flow disturbing surface.

The Thrombotic Response.

The acute thrombotic response. In humans (Chapters 6 and 7) large amounts of thrombotic material have been observed adhering to the metal wires within a few days, despite extensive anticoagulant therapy. Branching points especially, were abundantly covered by the thrombotic mass. The same probably occurs in the porcine coronary arteries as indicated by the intimal appearance at one week showing a thick layer of remnants of thrombus covering only the stent wires and not the adjacent "within stent" segments.

Thrombotic complications. In pigs, thrombotic occlusion seems to depend on the kind of material (chemical, electrical and surface properties) and the pharmacological treatment given (early) after implantation. Stainless steel (self-expanding device, Chapter 3) resulted in a 62% patency rate in coronary arteries at one week. This could be increased to 82% with aspirin (100 mg/d) and 100% with coumarin. When medication was stopped, one week after stenting, patency rates further decreased to 56, 75 and 88% respectively, at 12 weeks. The application of a fluorocarbon coating (Biogold[5]) finally resulted in a 100% patency rate, without any additional medication. The metal tantalum (balloon-expandable device, Chapters 2 and 5) yielded a 100% patency rate in healthy coronary arteries at 1 and 4 weeks, and a 92% patency rate in the vein-graft model at 4 weeks, without medication. The haemocompatibility of tantalum is probably due to the electrically insulating and chemically resistant tantalum-oxide layer[6], but might also be influenced by the fact that this stent has an open design (low metal to surface area). A study using the self-expanding stainless steel Gianturco-Roubin stent to test antithrombotic therapy, reports no difference in patency rates (controls were also 100% patent) and luminal narrowing following different pharmacological regimen[7]. The reason for the difference in thrombogenicity between the self-expanding Wallstent and the Gianturco Roubin stent might be influenced by both design and composition. The Gianturco Roubin stent has a considerably more open design than the Wallstent and thus less metal surface to induce thrombogenicity. Metal composition might also be of influence but the composition of the Gianturco Roubin stent is at present unobtainable.

Synthetic polymers are more versatile than metals for example with respect to variation in mechanical behaviour and possibilities for drug incorporation. This led us to testing the polyester polyethylene terephthalate (Dacron). A self-expanding device was

constructed (Chapter 4) and, for lack of a coronary delivery system, placed in femoral and carotid arteries. This material yielded a 88% patency rate at 4 weeks, without medication. We are convinced however that the material properties can be improved by different sterilization methods and more careful production of the stent.

The observation that the initial thrombotic response is mainly directed at the prosthesis surface, probably influenced by vascular components, again stresses the importance of a low prosthesis-covered to non-covered surface ratio. The ideal ratio would give an appropriate lumen geometry with a minimum of prosthesis-surface, resulting in a minimum of thrombus incorporation into the vessel wall.

Table I, Neointimal thickening in μm (range) at 1, 4 or 12 weeks after implantation, and % angiographic patency at follow-up. n.d. = no data available; e.a.h. = end-point attached heparin.

Stent	1 week	4 weeks	12 weeks	Patency	Ref.
Wiktor:					
coronary	56 (42-88)	139 (84-250)	n.d.	100%	Chapt. 2
Wallstent:					
peripheral					
+aspirin	80 (50-125)	n.d.	n.d.	100%	ref. 8
coronary	n.d.	n.d.	134 (39-413)	56%	Chapt. 3
+aspirin	n.d.	n.d.	101 (73-192)	75%	
+coumarin	n.d.	n.d.	143 (40-756)	88%	
+Biogold	n.d.	n.d.	113 (44-167)	100%	
Palmaz-S.:					
coronary	n.d.	263 (139-395)	n.d.	75%	ref. 9
+aspirin	n.d.	117 (82-188)	n.d.	57%	
+e.a.h.	n.d.	166 (126-204)	n.d.	60%	
+both	n.d.	109 (18-194)	n.d.	100%	
Polyester:					
peripheral	n.d.	114 (40-247)	n.d.	88%	Chapt. 4

The Process of Woundhealing.

It is now known (Chapter 2, [8]) that in pig arteries, stents are completely covered by endothelial like cells within one week, as observed by scanning and transmission electron microscopy. The intimal thickening consists of organized thrombus remnants overlaying the

stent wires. Within four weeks, these remnants are cleared and replaced by several layers of smooth muscle cells. During this interval, the intima increases in thickness (Table 1) as shown for the Wiktor stent. This process is probably similar for all stents in the healthy coronary circulation, as long as there is no excessive damage to the vessel wall[10]. In atherogenic swine models however, endothelialization was not complete until four weeks after stenting, although the stents were completely embedded in the vessel wall[7,11]. In these studies, intimal thickness increased to a maximum at two to four weeks.

Woundhealing in vein grafts seems even slower. In pig vein-grafts, not all stents are completely embedded in the vascular wall at one month, although the endothelial lining had regenerated. In the human grafts all stents at three months however, were completely embedded in the vascular wall and covered by endothelium. In some places only a very thin layer of fibrous tissue was found. Delayed thrombus clearance was observed in both the human and porcine vein grafts (Chapters 5 and 6). In pigs, unorganized thrombus was observed in close contact with the prosthesis at one month after implantation in the graft segment only. The adjacent carotid artery showed no delayed healing, responding to stent implantation similar as coronary arteries. Although these observations are indicative of a slow healing wound, we cannot exclude that we are merely dealing with an increased thrombotic response in the graft segments. In humans, thrombus remnants were present as fibrin and foamcells as long as 10 months after stent implantation. The main constituents of the intimal thickening in both pigs and humans were smooth muscle cells in a collagen rich matrix.

Restenosis or excessive intimal hyperplasia within the stent was observed in several patients as described in Chapter 6. The intimal thickening in these cases consisted of smooth muscle cells within an extensive collagenous matrix. Foamcells and extracellular lipids were not observed in these areas. These findings are in agreement with human coronary restenosis specimen obtained by atherectomy both after stenting and PTCA alone[12]. Although a clear indication of the cause for this thickening was not histologically apparent, there are indications that extensive vascular damage may be an important factor. Damage to the vascular wall can be assessed histologically. In a model of oversized stent placement, the vascular damage was graded according to a set of criteria[10] and was found to correlate linearly with the amount of intimal thickening. Another indication of damage induced excessive intimal hyperplasia is the fact that oversized stent placement in humans[13] is correlated with restenosis.

Chronic Injury.

As mentioned, implantation of stents is always accompanied by damage to the vascular wall. In order to determine wether or not stents can also induce chronic injury, the data from

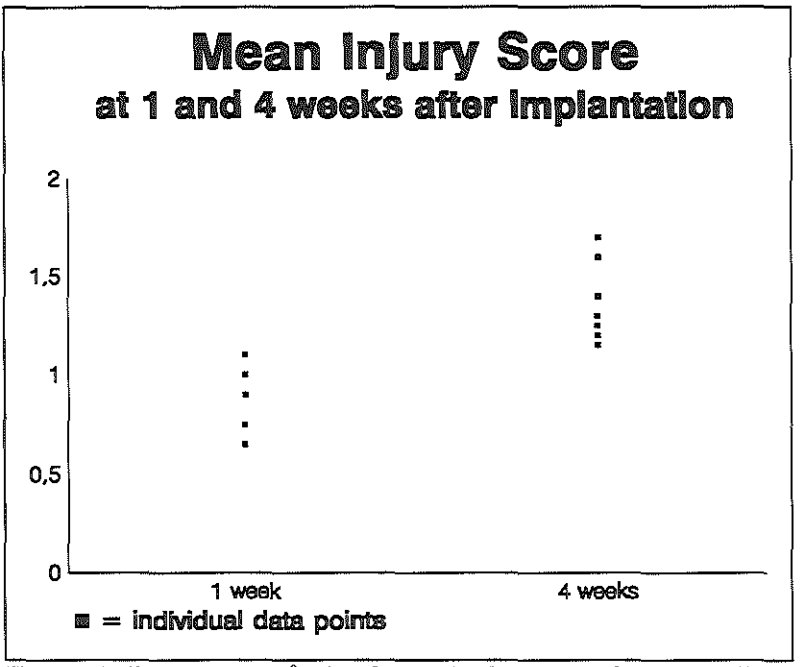

Figure 11 The injury score[9]: denudation (0); laceration of: Internal Elastic Membrane (1); media (2); and External Elastic Membrane (3).

Chapter 2 were used to grade the vascular damage after stenting according to a list of criteria[10]. A significant difference in mean injury was found between Wiktor stented coronary arteries at 1 and at 4 weeks, expressing itself as more frequent medial laceration (Figure 1).

The idea is that movement of the rigid implant in a pulsating artery is responsible for this phenomenon, perhaps facilitated by temporary changes in the vessel wall due to the healing response. It is not known wether this specific phenomenon is typical for balloon expandable stents (or perhaps the Wiktor stent), or if it also occurs in self-expanding stents. Self-expanding stents constantly exert an outward pressure which could very well induce chronic mechanical injury other than the acute medial impression. If, however they were as compliant as a normal artery (i.e. similar modulus of elasticity), the damage might be less.

Another interesting observation mentioned in Chapter 4 is the possible change in smooth muscle cell expression of the cytoskeletal protein Desmin, whereas no visible change was observed in expression of smooth muscle cell specific α-actin. Further testing has confirmed this change in Desmin-expression for all polyester stented arteries from that study. This might indicate a change in smooth muscle cell phenotype, perhaps with respect to its contractile function. It has to be determined, however, if this phenomenon occurs in all stented arteries or wether it is specific for peripheral arteries or even for this polyester stent.

Stenting and Accelerated Lipid Accumulation.

The process of healing after vascular injury probably elicits a similar healing response for different intervention techniques[14,15]. There are features in the case of stent implantation however that are unique as a thrombogenic and possibly chronically injuring permanent foreign body is introduced in the vessel wall. This might be associated with an important observation from Chapter 6, i.e. the presence of large accumulations of foamcells and extracellular lipids in the vicinity of the stent.

It is known that large amounts of thrombus adhere to the metal wires of the prosthesis. The monocytes (macrophages) from this thrombus, in combination with thrombin activated platelets are a perfect feeder layer for the accumulation of foamcells found three months after stenting (Chapter 6)[16]. The large pools of extracellular lipids, found after six months, might be released by macrophages that have reached the end of their life span, thereby leaving their contents in the vicinity of the stent.

It is also possible that we are dealing with lipids from a pre-existing plaque. This would imply that the stent wires lacerated or ruptured the thin fibrous cap covering the atheromatous plaque. Woundhealing after stent implantation might re-create a fibrous cap and give the suggestion of a stent related phenomenon, as the lipids are found within the new intimal thickening.

The presence of fibrin near the stent wires, deep in the atheromatous plaque, indicate that the wires were in contact with flowing blood for a considerable amount of time and points in the direction of either delayed plaque rupture or accelerated atherosclerosis (i.e. de novo lesion).

Vascular (dys)Function.

As shown in Chapter 8, de-endothelialized porcine coronary arteries exhibit an increased permeability for angiotensin (ANG)-I and/or II (1kDa), as indicated by an increased breakdown of ANG-II when compared to controls. Also stented coronary arteries show this increased ANG breakdown, which indicates a problem at the level of endothelial permeability. This hypothesis was tested for both balloon-expandable and self-expanding stents by studying in vivo the penetration of Evans Blue (EB, 1 kDa) and EB coupled to serum albumin (70 kDa)[17].

Macroscopical analysis revealed penetration of EB into the vessel wall up to 12 weeks after stenting, specifically in the tissue covering the metal stent wires. When EB was coupled to albumin however no penetration was found at either time point. Microscopical analysis revealed that all stents were completely endothelialized, apparently without any missing cells.

This indicates that stenting decreases long-term endothelial integrity, but leakage is restricted to smaller molecules such as EB and ANG-I and -II.

Future Goals.

Strong versus soft stents. As mentioned, stents are capable of inducing chronic injury. One way to reduce this injury is to create strong devices to treat resilient or tough lesions, while softer, more compliant stents could be used to tack back dissection flaps for the improvement of PTCA results or to prevent threatened or acute closure. Synthetic polymers could be used for the construction of self-expanding stents that showed hysteresis, i.e. deformation or shape change, resulting in loss of outward pressure. Reducing the outward pressure will reduce barotrauma and therewith a possible stimulant for intimal hyperplasia[13,10].

Biodegradable stents. An elegant solution to the problem of long-term presence of these prostheses is the use of biodegradable materials. Until now a suitable candidate has not been found. Several biodegradable synthetic polymers have been tested (e.g. polyglycolic-polylactic-acid copolymers[18]), and most elicit a strong inflammatory response and induce a considerable amount of intimal hyperplasia. The degradation products themselves are not only a source for possible cytotoxic substances but the fast biodegradation is also likely to give a stronger inflammatory response as all degradation products have to be cleared. This is an unfavourable situation, as it might interfere with woundhealing and subsequently endothelialization. Any delay in the latter may increase thrombotic complications. The search for biodegradable synthetic polymers with a "clean" degradation profile and a limited response of intimal hyperplasia have to be continued.

Drug releasing stents. Another feature of stents is the possibility to make use of these prostheses as a vehicle for local drug delivery. The possibility of local drug delivery is attractive as it enables the use of high local drug concentrations that might otherwise have adverse systemic effects. The perfect prosthesis would elute antithrombotic agents and drugs to enhance woundhealing in the acute phase (1 to 2 weeks) and an agent to prevent excessive intimal hyperplasia (restenosis) after woundhealing has been completed. Although the technology is available for creating prostheses with sequential drug delivery, the pharmacological knowledge to reduce restenosis is not available which makes it the achilles heel for treatment or prevention of restenosis.

Vascular function. It is clear that the first generation metal stents do not fulfill the criteria mentioned in Chapter 1. In order to make the perfect stent, more information is needed on the impact of stents on endothelial and smooth muscle cell function. These data can then be used to design a stent with an apropriate combination of mechanical support and drug release.

Conclusion.

In order to gain insight in the effect of stenting, the process of woundhealing was studied as well as the short- and long-term effects of these endovascular prostheses on the vascular wall. From this thesis and in conjunction with the literature, several statements can be made:

1. The stainless steel Wallstent is more thrombogenic than the tantalum Wiktor stent and the polyester stent in normal porcine arteries.

2. In normal porcine coronary arteries the thrombotic reaction to stainless steel can be prevented by coumadin, the fluorocarbon Biogold coating, and by a combination of aspirin treatment with an endpoint attached heparin coating.

3. Endothelial damage after stenting seems restricted to areas adjacent to and in direct contact with the prosthesis.

4. Woundhealing after stenting of porcine arteriovenous grafts seems delayed in comparison to normal and hypercholesterolemic porcine coronary arteries.

5. In human saphenous vein coronary artery bypass grafts, stenting seems associated with prolonged presence of thrombus remnants, and accelerated lipid accumulation.

6. Stents are capable of inducing long-term vascular damage resulting in both medial damage, a possible phenotypic change of medial smooth muscle cells and increased endothelial permeability.

Although the present generation of stents do not fulfil the criteria mentioned in Chapter 1, some insight has been gained into the effects of stenting and the resulting vascular damage. Still, stents hold a promise for prevention or treatment of restenosis which, with future research, will hopefully yield an endovascular prosthesis with a better performance and suitable for adapting to a wide range of lesions.

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Summary

Endovascular prostheses, also called stents, have been developed as an adjunct to or as a replacement for conventional balloon angioplasty. They act as an inner mechanical support for the vessel wall. For instance in case of acute problems such as dissection or recoil of the lesion after conventional balloon angioplasty. They are also implicated in preventing restenosis, the renewed narrowing of a previously treated lesion. Although these prostheses are clinically used, not much is known about the short- and longterm effect of stenting.

To gain insight in the effect of these metal prostheses on the vascular wall, the healing response after stent implantation was studied as well as the long term effect of these permanently present "foreign bodies". Several techniques were used in this study. Quantitative angiography revealed the luminal diameter before, during, and at several time instances after stent implantation. This technique enables the measurement of luminal narrowing as a function of time. Histological techniques were used to characterize the vessel wall. The angiotensin metabolism of the stented vessel wall was studied as a physiological parameter.

In Chapter 1, an overview is given of stent history. The prerequisites for an endovascular stent and possible improvements in relation to the clinically important problems are discussed.
In Chapter 2, the experimental evaluation of a balloon-expandable tantalum Wiktor stent is discussed. The vessel wall of pig coronary arteries was studied at 1 and 4 weeks after stent implantation.
In Chapter 3, the experimental evaluation of the self-expanding stainless steel Wallstent is discussed. The effect of anticoagulant therapy on acute occlusion and intimal hyperplasia was compared to a polymer coating on the Wallstent. The polymer coating was aimed at increasing biocompatibility. The vessel wall was studied at 12 weeks after implantation in pig coronary arteries.
In Chapter 4, the histological features of the vessel wall are discussed after implantation of a synthetic stent constructed of the polyester polyethylene terephthalate, studied in porcine peripheral arteries.
In Chapter 5, the reaction of the vessel wall is described after stenting with the balloon expandable stent discussed in Chapter 2, but now in an animal model for saphenous vein bypass grafting. The results are discussed in comparison with balloon angioplasty or in absence of treatment.
In Chapter 6, the vessel wall of human saphenous vein coronary artery bypass grafts is described after stenting with the self expanding Wallstent discussed in Chapter 3. The vessel wall is described at 3 days to 320 days after stent implantation.
In Chapter 7, stenting is discussed as an adjunct to balloon angioplasty, and gives an overview of animal experimental data and human histological data.
In Chapter 8, the first indication of chronically altered characteristics of the porcine coronary arteries after stent implantation is described.
In Chapter 9, a summary is given of the results and conclusions. Additionally, the latest experimental data are discussed concerning chronically changed characteristics of the vessel wall after stent implantation.

Samenvatting

Endovasculaire prothesen, ook wel stents genaamd, zijn ontwikkeld als aanvulling en eventuele vervanging van conventionele ballon angioplastiek. Deze stents zijn bedoeld om inwendig mechanische ondersteuning aan de vaatwand te verlenen. Bijvoorbeeld in geval van acute problemen na ballon dilatatie zoals het scheuren van het behandelde bloedvat (dissectie), of het niet of nauwelijks opgerekt blijven van de behandelde vernauwing (recoil). Tevens zouden zij een rol kunnen spelen in het voorkomen van restenose, het hernieuwd vernauwd raken van een bloedvat op langere termijn. Hoewel deze prothesen reeds in de kliniek worden gebruikt, is nog vrij weinig bekend over het effect van stents op korte en langere termijn.

Teneinde inzicht te verkrijgen in het effect van deze metalen prothesen op de vaatwand, is onderzoek verricht naar het herstel van de vaatwand na plaatsing van de prothese, en op lange termijn de effecten van deze permanent aanwezige metalen "vreemde lichamen". Hiertoe is gebruik gemaakt van verschillende technieken. Kwantitatieve coronaire angiografie werd gebruikt om de diameter van het bloedvat te meten vóór, tijdens, en op verschillende tijdstippen ná het plaatsen van de prothese. Zo is het mogelijk om het ontstaan van vernauwingen of afsluitingen in de tijd te volgen. Verder is gebruik gemaakt van verschillende histologische technieken om de vaatwand te karakteriseren. Als fysiologische parameter is in vitro het angiotensine metabolisme van de gestente vaatwand onderzocht.

In hoofdstuk 1 wordt een overzicht gegeven van de geschiedenis en het ontstaan van het fenomeen stent. Vervolgens worden de eisen voor de endovasculaire stent besproken en mogelijkheden ter verbetering van de prothese in relatie tot klinisch aangetoonde problemen.
In hoofdstuk 2 wordt de experimentele evaluatie besproken van de ballon-expandeerbare Wiktor stent die geconstrueerd is van het metaal tantalum. De vaatwand is bestudeerd 1 en 4 weken na stent implantatie in varkens coronaire arteriën.
In hoofdstuk 3 wordt de experimentele evaluatie van de zelf-expanderende Wallstent besproken die geconstrueerd is van roestvrij staal. Het effect van verschillende anti-stollings therapie op acute afsluiting en het lange termijn gedrag van de prothese is bestudeerd in vergelijking met dezelfde stent maar dan bedekt met een laagje polymeer. Deze coating is bedoeld om het biocompatibiliteitsprofiel van de stent te verbeteren.
In hoofdstuk 4 wordt de histologie beschreven van de vaatwand na implantatie van een geheel synthetische stent, gefabriceerd van het polyester polyethyleen terephthalaat.
In hoofdstuk 5 wordt de reactie van de vaatwand beschreven na stenting met de ballon-expandeerbare tantalum stent die reeds beschreven werd in hoofdstuk 2, maar nu in een diermodel voor veneuze bypass grafts. De resultaten worden vergeleken met het plaatsen van meerdere stents, met alleen ballon-angioplastiek, en met resultaten zonder behandeling.
Hoofdstuk 6 beschrijft de vaatwand van humane veneuze aortocoronaire bypass grafts behandeld met de zelf-expanderende Wallstent, reeds beschreven in hoofdstuk 3. Materiaal was beschikbaar vanaf 3 tot 320 dagen na het implanteren van de prothese.
Hoofdstuk 7 bespreekt stent implantatie als aanvulling op ballondilatatie en geeft een overzicht van de dierexperimentele en klinische gegevens.
Hoofdstuk 8 beschrijft de eerste aanwijzing van chronisch veranderde eigenschappen van de gezonde vaatwand, geïnduceerd door de stent.
Hoofdstuk 9 geeft een samenvatting van de gevonden resultaten en de daaruit volgende conclusies. Tevens worden de laatste experimentele gegevens besproken met betrekking tot chronisch veranderde eigenschappen van de vaatwand na stent implantatie.

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Curriculum vitae

Helena Margaretha Maria van Beusekom was born on May 9 1964 in Leeuwarden. She started her study in medical biology at the Free University in Amsterdam in 1982 where she specialized in molecular microbiology (Dr. B. Oudega, Free University Amsterdam) and celbiology (Prof. Dr. J.F. Jongkind, Erasmus University Rotterdam). Since 1989 she works as a scientist at the department of Experimental Cardiology of the Thoraxcenter, Erasmus University Rotterdam.

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