Comparison of diamond-like carbon-coated nitinol stents with or without polyethylene glycol grafting and uncoated nitinol stents in a canine iliac artery model

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Objective: Neointimal hyperplasia is a major complication of endovascular stent placement with consequent in-stent restenosis or occlusion. Improvements in the biocompatibility of stent designs could reduce stent-associated thrombosis and in-stent restenosis. We hypothesised that the use of a diamond-like carbon (DLC)-coated nitinol stent or a polyethylene glycol (PEG)-DLC-coated nitinol stent could reduce the formation of neointimal hyperplasia, thereby improving stent patency with improved biocompatibility.
Methods: A total of 24 stents were implanted, under general anaesthesia, into the iliac arteries of six dogs (four stents in each dog) using the carotid artery approach. The experimental study dogs were divided into three groups: the uncoated nitinol stent group ($n=8$), the DLC-nitinol stent group ($n=8$) and the PEG-DLC-nitinol stent group ($n=8$).
Results: The mean percentage of neointimal hyperplasia was significantly less in the DLC-nitinol stent group ($26.7\pm7.6\%$) than in the nitinol stent group ($40.0\pm20.3\%$) ($p=0.021$). However, the mean percentage of neointimal hyperplasia was significantly greater in the PEG-DLC-nitinol stent group ($58.7\pm24.7\%$) than in the nitinol stent group ($40.0\pm20.3\%$) ($p=0.01$).
Conclusion: Our findings indicate that DLC-coated nitinol stents might induce less neointimal hyperplasia than conventional nitinol stents following implantation in a canine iliac artery model; however, the DLC-coated nitinol stent surface when reformed with PEG induces more neointimal hyperplasia than either a conventional or DLC-coated nitinol stent.

Introduction

Endovascular treatment has been established for iliac or femoropopliteal artery occlusive disease [1–4]. Prevention of restenosis after endovascular treatment constitutes a significant challenge for the interventional radiologist, especially in long, heavily calcified and/or chronically occluded lesions [5]. Given their elastic properties, improved radial strength, ability to recover from being crushed and reduced foreshortening, nitinol stents have been proven to be a clinically effective therapeutic option for the treatment of iliac or femoropopliteal artery occlusion [3, 5, 6].

Nonetheless, excessive neointimal hyperplasia leading to in-stent restenosis still remains a major problem [5, 7] and can occur with nitinol stents in 20–40% of patients as long as 2 years after stenting [4, 8]. Improving the biocompatibility of stent designs could reduce stent-associated thrombosis and in-stent restenosis [9]. Thus, in order to both improve the mechanical properties and to provide sufficient radial strength and biocompatibility, a metallic stent has been functionally coated with various materials such as carbon [10, 11], silicon carbide [12] or tantalum [13]. Among these materials, a diamond-like carbon (DLC) stent coating has recently been introduced as a new stent design with improved biocompatibility; the coating reduces contact with the biomaterial, reduces metal ion release and decreases thrombogenicity [14–17]. In addition, a previous *in vitro* study [18] showed that grafting of polyethylene glycol (PEG), a highly biocompatible and hydrophilic polymer, onto a DLC-coated nitinol stent had the added benefit of enhancing the biocompatibility over that of uncoated nitinol and DLC-coated nitinol stents. Therefore, we hypothesised that the use of a DLC-coated nitinol stent or a PEG-DLC-coated nitinol stent could reduce the formation of neointimal hyperplasia, thereby improving the patency of stents with

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improved biocompatibility. In the current study, we evaluated and compared the efficacy of uncoated nitinol stents, DLC-coated nitinol stents and PEG-DLC-coated nitinol stents for reducing neointimal hyperplasia formation in a canine iliac artery model.

Methods and materials

A schematic illustration of the three stent types is shown in Figure 1.

DLC-coated stent

The self-expandable vascular nitinol stent (MeKo, Germany) of 6 mm in diameter (20% oversize compared with the diameter (4.8 mm) of a canine iliac artery) and 15 mm in length was formed by laser machining of a superelastic nitinol tube (SE 508 tube, Nitinol Devices and Components Inc.,). Prior to DLC coating, the uncoated nitinol stents were cleaned with methyl alcohol in an ultrasonic bath and were blown dry using nitrogen gas. The hybrid ion beam system was used for the surface pre-treatment and the DLC coating. The substrate holder was rotated at a speed of 3.33 rpm for uniform pre-treatment and coating of the entire surface of the stent. Base pressure in the chamber was less than 5×10^{-3} Pa and a radiofrequency (RF) bias voltage was applied to the substrate holder. Ar (argon) ion pre-cleaning and deposition of an amorphous Si (silicon) layer were performed to improve the interfacial adhesion strength between the DLC and the nitinol stent surface. Detailed conditions for the pre-treatment and DLC coating are referred to elsewhere [10]. The thickness of the DLC film for our experiment was 120 ± 5 nm and measured by a surface profilometer (alpha-step 200, TENCOR Instruments).

PEG coating conditions

Before PEG grafting on the surface of DLC-coated nitinol stents, steps were taken to remove the impurities and oxide on the surface of the stents. Samples were dipped in distilled water and acetone for 5 min before being immersed for 5 min in a mixture (1:1:5 v/v/v) of 25% ammonium hydroxide, 30% hydrogen peroxide and distilled water at 80°C. The samples were then sonicated for 30 min at room temperature in a solution of butanol and water (9:1 v/v). After rinsing the samples three times in ethanol and triple-distilled water, they were vacuum-dried for 24 h to obtain an oxidised, DLC-coated nitinol stent. These samples were immersed in 0.2 g of isocyanated PEG in 2 ml of toluene and 0.004 ml of stannous octoate at 40°C for 24 h to produce PEG-grafted nitinol-DLC stents [18].

Animal preparation

All experiments were performed in accordance with the National Institutes of Health guidelines for humane handling of animals and were approved by the Committee of Animal Research at our institute. Under general anaesthesia, a total of 24 stents were implanted, according to a fixed protocol, into the iliac arteries of six dogs (four stents in each dog) (with a weight of >25 kg). The animals in this experimental study were divided into three groups: the uncoated nitinol stent group ($n=8$), the DLC-nitinol stent group ($n=8$) and the PEG-DLC-nitinol stent group ($n=8$).

Stent placement procedure

After fasting for at least 8 h, animals were immobilised in the supine position. Dissection was made to access the
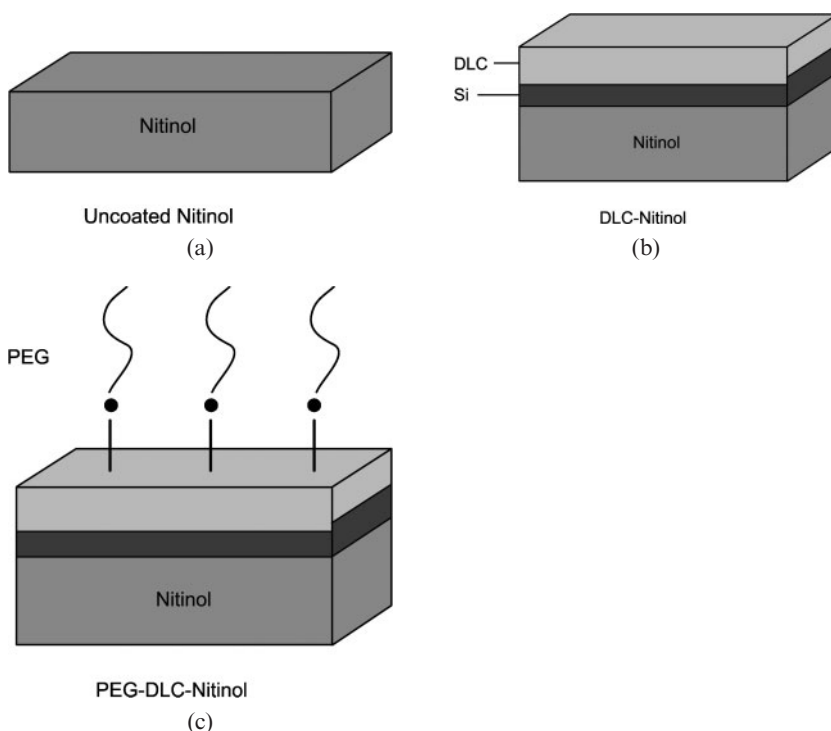

Figure 1. Schematic drawings of the three stent types. (a) Uncoated nitinol stent. (b) Diamond-like carbon (DLC)-coated nitinol stent. (c) Polyethylene glycol (PEG)-DLC-coated nitinol stent. Si, silicon.

right carotid artery and the vessel was isolated from the surrounding tissue. After puncture of the exposed right carotid artery, a guide wire (Terumo, Tokyo, Japan) and vascular catheter were advanced into the abdominal aorta under fluoroscopic guidance; angiography was performed in order to identify the vascular anatomy. Either two bare nitinol, two DLC-nitinol or two PEG-DLC-nitinol stents were placed in each limb of each dog. One stent was placed above (cranial position) and the other one below (caudal position) the origin of the deep circumflex iliac artery. In each dog, two kinds of stents were placed face to face (*e.g.* two bare nitinol stents were located in one limb and two DLC-nitinol stents were located in the other limb at the same level opposite to the bare nitinol stents; Figure 2). In each of the three groups, the basic distribution of bare nitinol, DLC-nitinol or PEG-DLC-nitinol stents was equal. After stent placement, an abdominal aortogram was obtained to evaluate the position and expansion of the implanted stent. Neither anticoagulant nor antiplatelet therapy was given before or after stent placement.

Follow-up and histological examination

After stent placement, all animals were maintained on their usual diet for 6 weeks. After 6 weeks, all dogs were sacrificed by an overdose of xylazine hydrochloride. Just before the sacrifice, angiography was performed to evaluate the position or possible thrombotic occlusion of the stents. The iliac arteries, distal abdominal aorta and proximal segments of the femoral arteries were removed and fixed in buffered formalin at physiological pressure. After fixation, the specimens were embedded in methyl methacrylate (Polyscience Inc., Warrington, PA). The stented area was cross-sectioned at three levels: the proximal end, middle and distal end of the stented segments. Sections of 40–50 μm were obtained and photographed under a light microscope.

Morphometric analysis of the neointima according to the histology slides was carried out by two independent observers. The digitised images were taken through an image acquisition system linked to a light microscope (BX51, Olympus Optical Co., Tokyo, Japan) at $40\times$ magnification and were analysed with Motic Images Plus software 2.0 ML (Ted Pella, Inc., CA). The area within the internal elastic lamina was considered the normal reference lumen area. Neointimal hyperplasia was determined by subtracting the patent lumen area from the area defined by the internal elastic lamina. The percentage of neointimal hyperplasia was calculated as $100\times(1-[\text{stenotic lumen area/normal reference lumen area}])$. Two measurements were made for three serial cross-sections from each stent and their mean values were obtained.

Statistical analysis

The Mann–Whitney *U*-test was performed to evaluate the significance of the microscopic findings. The SPSS version 14.0 statistical package (SPSS, Chicago, IL) was used to perform the analyses. A two-sided *p*-value <0.05 was considered to indicate a statistically significant difference.

Results

All stents were successfully placed in iliac arteries without any procedure-related complications. All six dogs survived until they were euthanised 6 weeks after stent placement. Angiography just before that showed no evidence of thrombotic occlusion or of stent migration.

Gross inspection of the periprosthetic soft tissue appeared similar for all stents. The mean percentage of neointimal hyperplasia was significantly greater in the PEG-DLC-nitinol ($58.7\pm24.7\%$) stent group than in the nitinol stent group ($40.0\pm20.3\%$) ($p=0.01$). The mean percentage of neointimal hyperplasia was significantly less in the DLC-nitinol stent group ($26.7\pm7.6\%$) than in the nitinol stent group ($40.0\pm20.3\%$) ($p=0.021$) (Figure 3). Figure 4 shows the cross-sectional
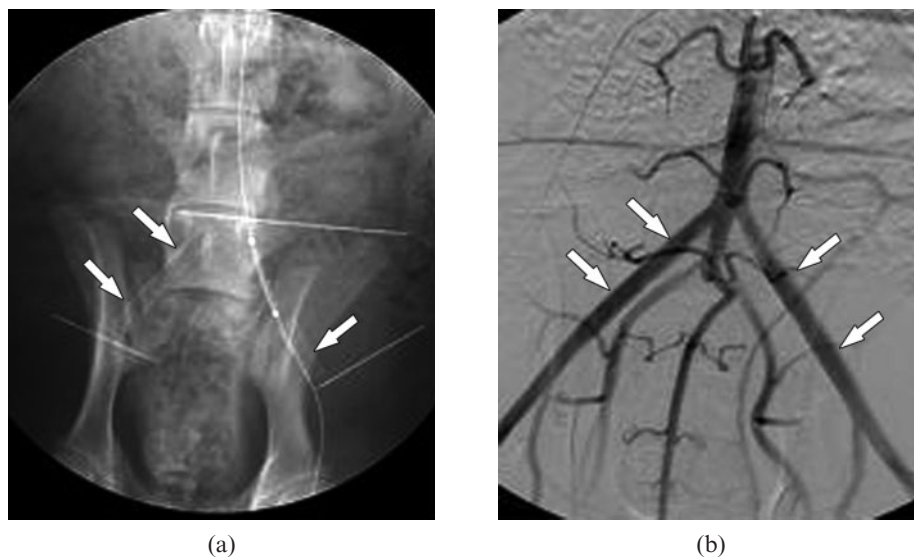

(a)   (b)

Figure 2. Stent placement procedure. (a) Stents (arrows) are placed in both iliac arteries. (b) Angiography immediately after stent placement (*n*=4, two stents in each iliac artery) shows good position and expansions of the stents (arrows).
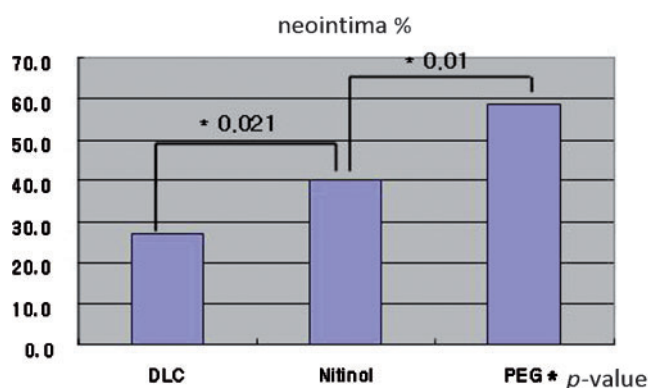
Figure 3. Percentage of neointimal hyperplasia in the three different stent groups. DLC, diamond-like carbon; PEG, polyethylene glycol.

photomicrographs of representative serial pathology specimens from the three groups. In all cases in the PEG-DLC-nitinol stent group, a large portion of the neointimal hyperplasia was due to the overproduction of collagen secreted by proliferated fibroblasts (Figure 4d).

Discussion

Restenosis caused by neointimal proliferation is the primary drawback of stent placement for peripheral arterial occlusive disease. The vast majority of restenoses occur within 3 months after stent placement [19]. Restenosis can be more problematic with infra-inguinal (30–50%) than with coronary stent implantation (25%) [19, 20]. The aetiology of neointimal hyperplasia resulting in in-stent restenosis can be a contact allergy or enhanced proliferative tissue response to nickel, chromate or molybdenum ions released from the stents [14, 15].

Given their reduced metal ion release and platelet activation, DLC-coated stents are considered to be a promising stent design because of their improved biocompatibility and decreased thrombogenecity. For example, Salahas et al [21] reported an observational prospective non-randomised study regarding DLC-coated stent placement in 196 patients in 236 significant *de novo* atheromatous coronary lesions. During hospitalisation and the 6 month follow-up after stent placement, there was no major cardiovascular event and the rates for target lesion revascularisation and for target vessel revascularisation were 5.6% and 7.65%, respectively. The authors concluded that placement of intracoronary DLC-coated stents is associated with high success rates, safety and efficacy, as seen both in the hospital and at the 6 month follow-up after the interventional procedure.

The first report on the application of DLC-coated stents in infra-inguinal lesions was published by Schaefer et al [14]. The preliminary results of the placement of bare nitinol stents coated with DLC in two patients with superficial femoral artery occlusive disease was promising: in both these cases there was a primary patency rate of 100% 12 months after intervention. However, to our knowledge, there has been no study comparing DLC-coated stents and uncoated stents in the infra-inguinal artery.

This is the first experimental study comparing DLC-coated nitinol stents and uncoated nitinol stents in terms of neointimal hyperplasia on histological examination in an animal model. Our experimental study obtained a significantly lower percentage of neointimal hyperplasia in the DLC-coated stent group ($26.7 \pm 7.6\%$) than in the uncoated nitinol stent group ($40.0 \pm 20.3\%$) ($p=0.021$), as seen on the 6 week follow-up histological examination.

In a previous *in vitro* study [18], PEG-DLC-nitinol stents showed less protein adsorption and platelet adhesion and, therefore, improved biocompatibility. Thus, we anticipated that PEG-DLC-nitinol stents might have less neointimal hyperplasia after stent implantation than conventional nitinol stents or DLC-nitinol stents in our canine iliac artery model. Unexpectedly, the mean percentage of neointimal hyperplasia was significantly greater in the PEG-DLC-nitinol stent group ($58.7 \pm 24.7\%$) than in the nitinol stent group ($40.0 \pm 20.3\%$) or in the DLC-nitinol stent group ($26.7 \pm 7.6\%$). The important problem we found with PEG-DLC-nitinol stents is that they stimulate the proliferation of fibroblasts and thus create intraluminal stenosis because of the overproduction of collagen secreted by the fibroblasts. Another explanation for the disappointing results with PEG-DLC-nitinol stents in the present study might be that the PEG-coated surface could increase the surface roughness more than the DLC coating. It is well known that a rough surface can increase the chance of cell attachment.

Experimental studies regarding vascular stent placement in an animal model are usually performed via a femoral artery approach [9]. However, in our study, it was technically possible to place a stent in a canine iliac artery model using the carotid artery approach; this approach might be particularly useful for the precise placement of four stents per animal in the desired area.

The principal limitation of our study is that the stents were placed in normal canine iliac arteries, because an animal model of iliac stricture is difficult to create. Therefore, it is difficult to generalise the current results in iliac artery strictures of human patients. The other limitation was the small number of experimental animals, which could have decreased the statistical strength. However, we believe that our study can provide support for future, large or prospective clinical studies investigating the efficacy of DLC-coated stents in strictures of the infra-inguinal arteries.

Conclusion

DLC-coated nitinol stents might induce less neointimal hyperplasia than conventional nitinol stents after implantation in a canine iliac artery model. However, a DLC-coated nitinol stent surface reformed with PEG induces more neointimal hyperplasia than a conventional nitinol or DLC-coated nitinol stent.

Acknowledgments
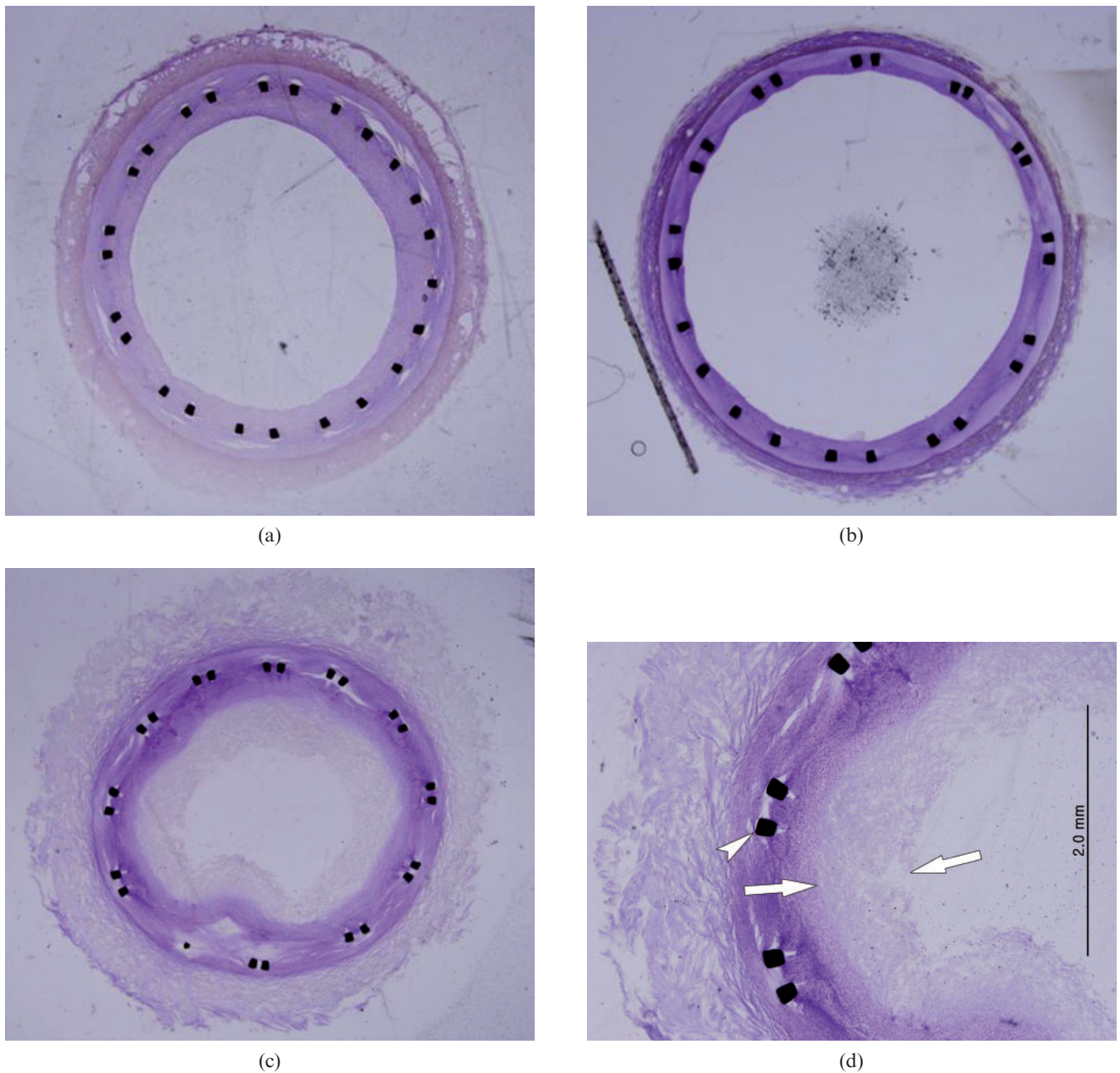
(a)

(b)

(c)

(d)

Figure 4. Cross-sectional photomicrographs of representative serial pathology specimens from the three groups. Low microscopic findings (a–c) show that the neointimal hyperplasia area was significantly less in the diamond-like carbon (DLC)-coated stent (b) compared with that in the nitinol stent (a) and was greater in the polyethylene glycol (PEG)-DLC-coated stent (c) than in the nitinol stent. A high-power microscopic image (d) for the PEG stent (c) shows that a large portion of the neointimal hyperplasia was caused by the overproduction of collagen (arrows) secreted by proliferated fibroblasts. Arrowhead: stent strut.

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