# Citric acid-based elastomers provide a biocompatible interface for vascular grafts

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**Abstract:** Prosthetic vascular bypass grafting is associated with poor long-term patency rates. Herein, we report on the mid-term performance of expanded polytetrafluoroethylene (ePTFE) vascular grafts modified with a citric acid-based biodegradable elastomer. Through a spin-shearing method, ePTFE grafts were modified by mechanically coating a layer of poly(1,8 octanediol citrate) (POC) onto the luminal nodes and fibrils of the ePTFE. Control and POC-ePTFE grafts were implanted into the porcine carotid artery circulation as end-to-side bypass grafts. Grafts were assessed by duplex ultrasonography, magnetic resonance angiography, and digital subtraction contrast angiography and were all found to be patent with no hemodynamically significant stenoses. At 4 weeks, POC-ePTFE grafts were found to be biocompatible and resulted in a similar extent of neointimal hyperplasia as well as leukocyte and monocyte/macrophage infiltration as control ePTFE grafts. Furthermore, POC supported endothelial cell growth. Lastly, scanning electron microscopy confirmed the presence of POC on the ePTFE grafts at 4 weeks. Thus, these data reveal that surface modification of blood-contacting surfaces with POC results in a biocompatible surface that does not induce any untoward effects or inflammation in the vasculature. These findings are important as they will serve as the foundation for the development of a drug-eluting vascular graft. © 2009 Wiley Periodicals, Inc. J Biomed Mater Res 93A: 314–324, 2010

**Key words:** ePTFE; prosthetic; bypass graft; polymer; endothelial cell; neointimal hyperplasia

## INTRODUCTION

Peripheral arterial disease (PAD) is a debilitating and disabling sequela of atherosclerosis. PAD is estimated to affect 8 million Americans and the prevalence is growing with the large increase of the aging population.[1] For patients with severe PAD, lower extremity bypass grafting often remains the only option for limb salvage. The gold standard conduit for infrainguinal bypass grafting is autologous vein. Although the patency for infrainguinal vein grafts remains ~70% at 5 years,[2] vein may not be available due to intrinsic venous disease or prior vein harvesting. In these cases, expanded polytetrafluoroethylene (ePTFE) grafts are the most commonly used alternative bypass conduit. However, the primary patency rates for infrapopliteal ePTFE bypass grafts are dismal. For example, when used in femoral-popliteal bypass grafting, the 3- and 5-year patency rates for ePTFE grafts are only 64% and 44%.[3] When used for infrapopliteal bypass grafting, the 1-, 2-, and 4-year patency rates for ePTFE grafts are 65%, 30%, and 12%, respectively.[4]

The etiology of long-term prosthetic bypass graft failure is commonly due to the development of neointimal hyperplasia at the distal anastomosis.[5–8] For some time now, surgeons have attempted to mitigate the problem of graft thrombosis with the oral administration of anticoagulant and anti-inflammatory drugs.[9] Furthermore, because of the lower long-term patency rates observed with prosthetic bypass grafting, surgeons have also developed intraoperative

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approaches to improve graft patency. These modifications are largely intended to decrease the compliance mismatch between the prosthetic graft and the native artery and include vein cuffs, vein patches, vein boots, and arteriovenous fistulas at the distal anastomosis.[10–14] However, despite these pharmacologic and surgical improvements, patency rates remain dismal, especially when the distal outflow is at or below the level of the popliteal artery. Thus, many researchers are investigating alternative strategies that involve surface modification of the graft's lumen to obtain a localized beneficial effect, thereby avoiding systemic side effects. To date, modifications of prosthetic grafts have been designed to present a more antithrombogenic surface, slowly release a drug, stimulate *in vivo* graft endothelialization, or improve the retention of *in vitro*-seeded endothelial cells following exposure to physiological blood flow and shear stress.[15] This research has seen modest success at preventing initial thrombus formation on the blood-contacting surface of the material; however, to date, no technology has successfully avoided thrombosis and the development of neointimal hyperplasia following prosthetic bypass grafting in large animal models of prosthetic bypass grafting or in prospective randomized clinical trials.

We previously reported the development, *in vitro* characterization, and short-term biocompatibility of biodegradable polyester elastomers based on citric acid.[16–18] These elastomers, referred to as poly(diol citrates) (PDC), demonstrated anticoagulant and antithrombotic properties *in vitro* and, hence, have the potential to improve blood-biomaterial interactions *in vivo*.[19] Furthermore, PDC can serve as a drug-delivery vehicle for compounds or proteins targeting the coagulation cascade of arterial injury response. The hemocompatible characteristics of PDC make them an attractive biomaterial choice for the surface modification of vascular grafts. Herein, we assess the 1-month hemocompatibility and intravascular tissue response to poly (1,8 octanediol citrate) (POC), a type of PDC, in a carotid artery bypass porcine model.

## MATERIALS AND METHODS

### Polymer synthesis

The synthesis and characterization of POC was previously published.[17,18] Briefly, equimolar amounts of citric acid and 1,8-octanediol were melted together at 160°C while stirring for 15 min. The temperature was subsequently decreased to 140°C and the mixture was stirred for 1 h. The prepolymer was purified by precipitation in water and freeze-dried for storage. The prepolymer was soluble in ethanol or 1,4-dioxane, which are less toxic than other commonly used solvents.

### Surface modification of the ePTFE graft with POC

The lumen of thin-wall stretch ePTFE grafts (6 mm inner diameter, Gore-Tex, W. L. Gore & Associates, Flagstaff, AZ) was modified by mechanically coating a POC layer onto the nodes and fibrils of ePTFE through a spin-shearing method as previously described.[16] Briefly, a 5 mm diameter glass rod was dipped into 10% POC prepolymer (pre-POC) solution in 1,4-dioxane and inserted horizontally into the motor of a mechanical stirrer (IKA-Werke GmbH & Co. KG, Eurostar ST P CV PS S1, Staufen, Germany). The pre-POC-coated glass rod was spun clockwise at 300 rpm for 2 min and an 8 cm long piece of ePTFE graft was placed concentrically over the spinning rod. The lumen of the graft was sheared against the spinning rod for 2 min by manually rotating the graft counterclockwise. The above procedure was considered to be one coating. To change the amount of POC deposited onto the graft, the above procedure was repeated three times (defined as three coatings). After air-drying, the pre-POC-coated ePTFE graft was put into an oven at 80°C for 2 days to obtain POC-ePTFE grafts. Grafts were sterilized via ethylene oxide exposure and degassing according to the manufacturer's instructions.

### Presurgical care and anesthesia

All animal procedures were performed by use of aseptic technique in accordance with the Northwestern University Animal Care and Use Committee. Domestic juvenile castrated male Yorkshire-Landrace pigs (Oak Hill Genetics, Ewing, IL) weighing 25–30 kg were utilized. Animals received antibiotic prophylaxis with one dose of cefazolin (25 mg/kg intramuscular [IM]) preoperatively and postoperatively. Aspirin (325 mg oral [PO]) was administered daily starting 5 days before surgery and continued throughout the entire postoperative course. On the day of surgery, the animals also received an aspirin suppository (325 mg).

Preoperative analgesia and sedation included buprenorphine (0.01 mg/kg IM), acepromazine (0.15 mg/kg IM), ketamine (20 mg/kg IM), and atropine (0.05 mg/kg IM). After intubation, anesthesia was maintained with inhaled isoflurane (0.5–2.0%) delivered with 100% oxygen. Temperature, heart rate, respiratory rate, and oxygen saturation were monitored continuously and recorded every 15 min throughout the procedure.

### Surgical procedure

Details of the animal model have been previously published.[20] Briefly, animals were placed in the supine position, and their necks were shaved then prepped with betadine and alcohol (70%). Pigs received bilateral ePTFE bypass grafts ($n = 5$). Through a midline neck incision,

both right and left common carotid arteries (CCA) were exposed. Following heparin (150 U/kg intravenous [IV]) administration, the right CCA was occluded proximally and in the midsection with noncrushing vascular clamps. A longitudinal arteriotomy was made, and a 6 cm length of PTFE graft (6 mm thin wall stretch ePTFE, Gore, Flagstaff, AZ) was anastomosed in an end-to-side fashion with running 6–0 polypropylene suture. Care was taken to ensure that the ePTFE graft was anastomosed at a 45° angle with respect to the native artery, thereby dictating a standard arteriotomy length. Before completion of the proximal anastomosis, the native vessel was flushed and irrigated with heparinized saline (2000 U heparin per 1 L normal saline). After completion of the proximal anastomosis, flow was restored in the CCA for 5 min while the graft was clamped near the anastomosis. Next, the right CCA was occluded distally and in the midsection with noncrushing vascular clamps. The distal anastomosis was created in a manner similar to the proximal anastomosis. Just before its completion, the graft and native artery were vigorously flushed with heparinized saline. Once the distal anastomosis was completed, the distal arterial clamp was removed, restoring blood flow to the CCA through the ePTFE graft. The midsection vascular clamp was replaced with double ligation using 2–0 silk suture to simulate an occlusion. After completion of the right bypass graft, a second dose of heparin (75 U/kg IV) was administered, and the left bypass graft was created in a similar manner. After both bypass grafts were completed, meticulous hemostasis was achieved, and the incision was closed in multiple layers using absorbable suture. Animals were monitored until awake, alert, and sternal. Postoperative analgesia consisted of buprenex (0.01 mg/kg IM) given every 12 h for the first 48 h postoperatively.

## Magnetic resonance angiography

Magnetic resonance angiography (MRA) was performed to evaluate graft patency 3 weeks postoperatively. After sedation with acepromazine (0.15 mg/kg), atropine (0.05 mg/kg), and ketamine (20 mg/kg), intubation was performed and anesthesia was maintained with inhaled isoflurane (1–2%) delivered with 100% oxygen. MRA was performed using a time-resolved T1-weighted gradient echo pulse sequence. A time-series of 3D contrast-enhanced images were acquired with a gadolinium-based contrast agent (0.1 mmol/kg IV; Magnevist, Berlex, Princeton, NJ). Noncontrast angiograms were also performed using a time-of-flight imaging protocol.

## Duplex ultrasonography

Four weeks postoperatively, just before sacrifice, both ultrasonography and contrast angiography were conducted. Following exposure of both the right and left CCA and bypass grafts, intraoperative duplex ultrasonography was performed, obtaining B-mode images and Doppler velocity measurements including peak systolic velocity (PSV) and end diastolic velocity (EDV). For each side, measurements were obtained at the proximal CCA, proximal anastomosis, proximal graft, mid-graft, distal graft, distal anastomosis, and distal CCA. A significant stenosis was defined as PSV greater than two times the normal inflow artery velocity.

## Contrast angiography

After assessing the grafts with duplex ultrasonography, digital subtraction contrast angiography was performed percutaneously by accessing the common femoral artery and placing a 5 French (F) introducer sheath. Using a 0.035″ J-wire, a 5 F multisided-hole catheter was advanced into the aortic arch under fluoroscopy. An arch angiogram was obtained with contrast injection (20 mL, Omnipaque, Amersham, Piscataway, NJ) through the catheter. Each CCA was selected using an angled guide catheter and the J-wire, and angiograms were obtained of both CCA and ePTFE grafts using 10 mL of contrast media. Following angiogram completion, the animals were euthanized with pentobarbital (72 mg/kg).

## Tissue processing

The ePTFE bypass grafts and native artery extending 2 cm from each anastomosis were harvested *en bloc* and underwent *ex vivo* perfusion-fixation in 10% buffered formalin for 12 h. Next, the samples were dehydrated with a graded ethanol series (70%, 95%, 100%), followed by xylene, then cut into 1 cm sections, and placed in plastic tissue cassettes (Tissue-tek, Hatfield, PA). The samples were embedded in paraffin, and blocks were cut into 5 μm transverse sections and the sections were transferred onto slides.

## Tissue staining and morphometric analysis

Sections were examined histologically for evidence of neointimal hyperplasia using routine Hematoxylin and Eosin (H&E) staining. Digital images were collected with light microscopy using an Olympus BHT microscope (Melville, NY). Five equally-spaced sections throughout each proximal and distal anastomoses were analyzed for each bypass graft. Neointimal hyperplasia (area in millimeters) under the ePTFE graft was assessed using ImageJ software (National Institutes of Health, Bethesda, MD) and adjusted according to the circumference length of ePTFE on each cross section. To accurately compare the degree of neointimal hyperplasia from each anastomosis, the angle of embedding and cutting the block with respect to the angle of the ePTFE to the native artery was accounted for and controlled so that each block was similar, as changes to the block orientation can artificially underestimate or overestimate the degree of neointimal hyperplasia.

## Immunohistochemistry

For immunohistochemistry, 5 μm-thick sections were deparaffinized in xylene and rehydrated in graded alcohol.

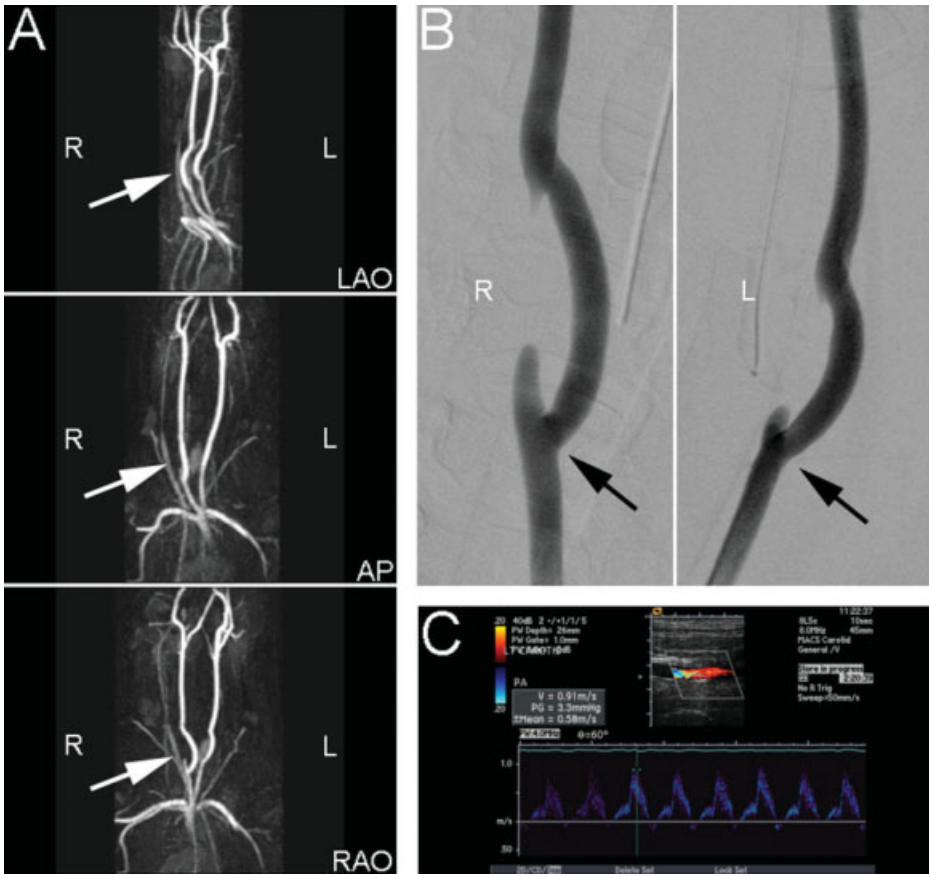


**Figure 1.** All POC-ePTFE grafts were patent at 28 days and without evidence of stenosis by noninvasive and invasive imaging modalities. A: Magnetic resonance angiogram of the pig carotid arteries in three different views, left anterior oblique (LAO), anterior-posterior (AP), and right anterior oblique (RAO). White arrows indicate the bypass grafts. Control ePTFE bypass grafts are on the right (R), and POC-ePTFE bypass grafts are on the left (L). B: Subtraction contrast angiography of the pig carotid artery bypass grafts. Right (R) represents a control ePTFE bypass graft and left (L) represents a POC-ePTFE bypass graft. Black arrows indicate the proximal anastomoses. C: Duplex ultrasonography image and associated velocity waveform of the proximal anastomosis of a carotid artery bypass graft. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

For the antigen retrieval process (required for detection of anti-human von Willebrand factor and mouse monoclonal MAC387), specimens were treated in a heated citrate buffer solution, pH 6.0 (Lab Vision, Fremont, CA) for 20 min. Immunohistochemistry was carried out using the HRP Polymer & DAB Plus Chromogen Ultravision LPValue Detection System (Thermo Scientific, Fremont, CA). Endogenous peroxidase activity was blocked by incubating the slides in a hydrogen peroxide blocking reagent (provided in kit) for 15 min. To block nonspecific background staining, specimens were incubated in Ultra V Block (provided in kit) for 10 min. Endothelial cells were detected with polyclonal rabbit anti-human von Willebrand factor (1:200, DAKO, Glostrup, Denmark) for 30 min at room temperature. Leukocytes were detected with purified mouse anti-pig CD45RA monoclonal antibody (1:200, BD Biosciences Pharmingen, San Jose, CA) for 60 min at room temperature. Macrophages/monocytes were detected with the mouse monoclonal MAC387 antibody (1:200, Abcam, Cambridge, MA) at 4°C overnight. Specimens were then incubated in the Value Primary Antibody Enhancer (provided in kit) for 20 min, rinsed, and incubated in Value HRP Polymer (provided in kit) for 30 min. The Specimens were then incubated in DAB Plus Chromogen and DAB Plus Substrate (provided in kit) for 3 min. Counterstaining was performed with Gill 2× Hematoxylin (Protocol, Fisher Scientific, Waltham, MA). For negative controls, antibody was omitted. To objectively assess the extent of monocyte and macrophage infiltration between groups, positive staining nuclei were counted in four high power fields per each section.

### Scanning electron microscopy (SEM)

Graft sections were fixed, dehydrated, and sputter-coated with gold before imaging on a Hitachi 3500 N at the EPIC facility of Northwestern University.

### Statistical analysis

Results are expressed as mean ± standard error of the mean. Differences between multiple groups were analyzed by use of one-way analysis of variance with the Student-Newman-Keuls post hoc test for all pairwise comparisons.

**TABLE I**
**Duplex Ultrasonography Velocity Measurements**

| Section | Control PSV (m/s) Mean ± SE | POC-ePTFE PSV (m/s) Mean ± SE | Control EDV (m/s) Mean ± SE | POC-ePTFE EDV (m/s) Mean ± SE |
|---|---|---|---|---|
| Proximal artery | 0.703 ± 0.12 | 0.958 ± 0.14 | 0.220 ± 0.05 | 0.224 ± 0.07 |
| Proximal anastomosis | 1.184 ± 0.30 | 1.669 ± 0.62 | 0.266 ± 0.07 | 0.441 ± 0.16 |
| Proximal graft | 0.881 ± 0.13 | 1.024 ± 0.13 | 0.164 ± 0.03 | 0.191 ± 0.05 |
| Mid graft | 0.810 ± 0.15 | 1.086 ± 0.18 | 0.139 ± 0.03 | 0.222 ± 0.07 |
| Distal graft | 0.834 ± 0.09 | 0.835 ± 0.15 | 0.200 ± 0.05 | 0.197 ± 0.05 |
| Distal anastomosis | 1.047 ± 0.18 | 1.385 ± 0.27 | 0.220 ± 0.07 | 0.266 ± 0.05 |
| Distal artery | 1.421 ± 0.24 | 1.674 ± 0.24 | 0.278 ± 0.09 | 0.293 ± 0.07 |

PSV, peak systolic velocity; EDV, end diastolic velocity; m/s, meters per second; SE, standard error; P, NS between treatment groups.

Differences between two groups were analyzed with the Student's *t*-test (SigmaStat, SPSS, Chicago, IL). Statistical significance was assumed when $p < 0.05$.

## RESULTS

### POC is nonthrombogenic and biocompatible in the vasculature at 28 days

Five adult male Yorkshire-Landrace pigs underwent bilateral ePTFE bypass grafting, each with a control ePTFE (right) and POC-ePTFE (left) bypass graft. At 21 days, all bypass grafts in both treatment groups were patent as assessed by MRA [Fig. 1(A)]. At 28 days, all bypass grafts in both therapy groups were patent as assessed by 2D subtraction contrast angiography [Fig. 1(B)], and duplex ultrasonography [Fig. 1(C)]. No significant stenoses were identified on any of the three imaging modalities. Furthermore, using duplex ultrasonography, peak and end diastolic velocities were obtained throughout the bypass grafts as well as in the proximal and distal native artery. No statistically significant differences were found between the velocities within each graft, as well as between the control and POC-ePTFE grafts (Table I). Following graft procurement, the proximal and distal anastomoses of the bypass grafts were assessed for the development of neointimal hyperplasia. POC-ePTFE grafts developed a similar degree of neointimal hyperplasia when compared with the control ePTFE grafts (Fig. 2), confirming the biocompatibility of POC.

### POC is present at 28 days

As POC is known to be biodegradable over time,[18] we assessed the bypass grafts for the presence of POC on the ePTFE after being in the circulation for 28 days. Following graft procurement, midsections of the graft were assessed using SEM. In control ePTFE grafts, the characteristic nodal and fibril distribution is seen, as well as scattered fibrous coagulum [Fig. 3(A)]. In grafts coated with POC, SEM images reveal residual POC coating, which was visible within a few cell-free areas of the graft [Fig. 3(B)]. Thus, the POC synthesis conditions used in this study do not lead to complete degradation within 4 weeks in the circulation.

### POC supports endothelialization *in vivo*

After graft harvesting, grafts from both treatment groups were assessed immunohistochemically for the presence of endothelial cells. POC supported endothelialization throughout all sections analyzed [Fig. 4(B)]. This endothelialization continued into the lumen of the graft material, far from the native artery. To further support this assessment, small sections of the graft material were processed for SEM. SEM confirmed the presence of an intact endothelial cell monolayer on both control and POC-coated ePTFE bypass grafts [Fig. 4(C)].

### POC does not induce inflammation *in vivo*

Immunohistochemical assessment of the anastomoses revealed similar patterns of CD45 positive leukocytes in control and POC-coated ePTFE bypass grafts [Fig. 5(A,B)]. In the native artery opposite the anastomosis, a similar degree of leukocyte infiltration was also observed between the two treatment groups [Fig. 5(C,D)]. With respect to monocyte and macrophage infiltration, a similar pattern was observed between the control and POC-ePTFE grafts; however, greater overall monocyte/macrophage infiltration was observed in both treatment groups when compared with that observed with leukocytes. A similar extent of monocyte and macrophage infiltration was observed in the neointima [Fig. 6(A–D)] and in the ePTFE graft material between the two

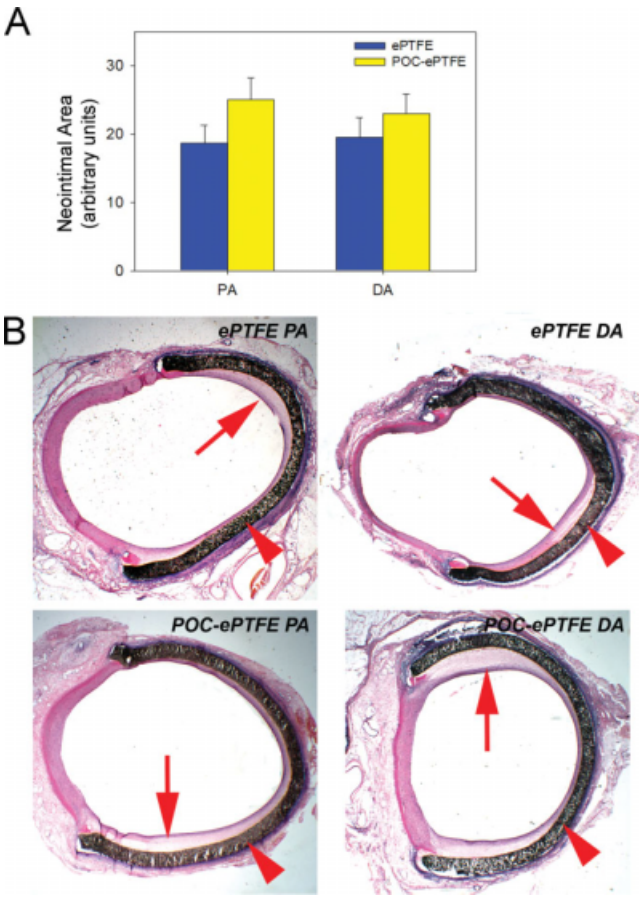


**Figure 2.** POC-ePTFE grafts produce a similar amount of neointimal hyperplasia when compared with control ePTFE grafts. A: Quantification of neointimal hyperplasia at the proximal anastomosis (PA) and distal anatomosis (DA) of the control ePTFE and POC-ePTFE bypass grafts. B: Hematoxylin and eosin stained cross sectional images of control ePTFE and POC-ePTFE bypass grafts at the PA and DA. Arrows indicate neointimal hyperplasia. Arrowheads indicate the graft material. 1× magnification. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

treatment groups [Fig. 6(E,F)]. Of note, infiltration of monocytes and macrophages into the prosthetic material was commonly observed in both treatment groups, whereas CD45 positive leukocytes were not observed in the interstices of the graft material.

## DISCUSSION

The ideal prosthetic graft should be nonthrombogenic, hemo- and bio-compatible at high and low shear stresses, have similar compliance to native vessels, and inhibit the development of neointimal hyperplasia. As the majority of new biomaterials have failed to replicate the mechanical and biological properties of blood vessels, researchers have turned to surface modification approaches to improve the safety and efficacy of current intravascular devices. Unlike other surface modification techniques, POC modification of ePTFE does not involve exposure to plasma glow discharge or other harsh solvent treatments. Mechanical spin shearing is able to comprehensively coat the nodes and fibrils within the ePTFE graft lumen. We have previously reported the *in vitro* and short-term hemocompatibility characterization of POC.[16,19] Furthermore, the modification of ePTFE grafts with POC improved endothelial cell adhesion, reduced platelet adhesion *in vitro*, and did not affect graft compliance.[16,21] In the study described herein, we hypothesized that the mechanical and interfacial properties of POC would make it a viable candidate material for the surface modification of vascular grafts. Although the exact mechanisms for these characteristics are under study, the possibility to use POC as a base material to engineer a functional endothelium *in vitro* and/or locally deliver drugs in the vasculature prompted a longer term evaluation of its intravascular biocompatibility.

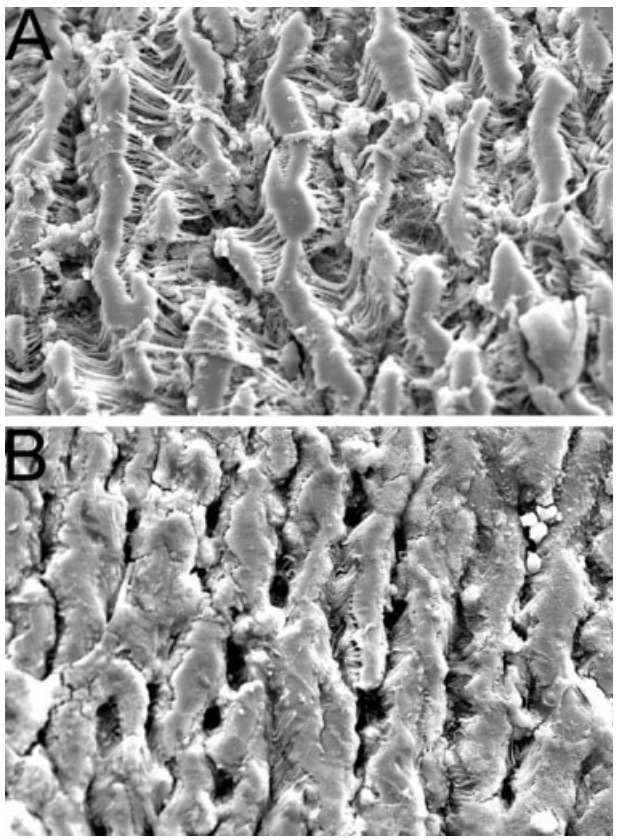


**Figure 3.** The POC coating is still present on the ePTFE grafts at 28 days. Scanning electron microscopy of a midsection of a A: control ePTFE and B: POC-ePTFE bypass graft harvested after 28 days in the porcine carotid artery circulation. Note the persistent POC coating on the POC-ePTFE bypass graft at 28 days as well as the characteristic nodal and fibril pattern of the ePTFE material. 50 μm scale bar.

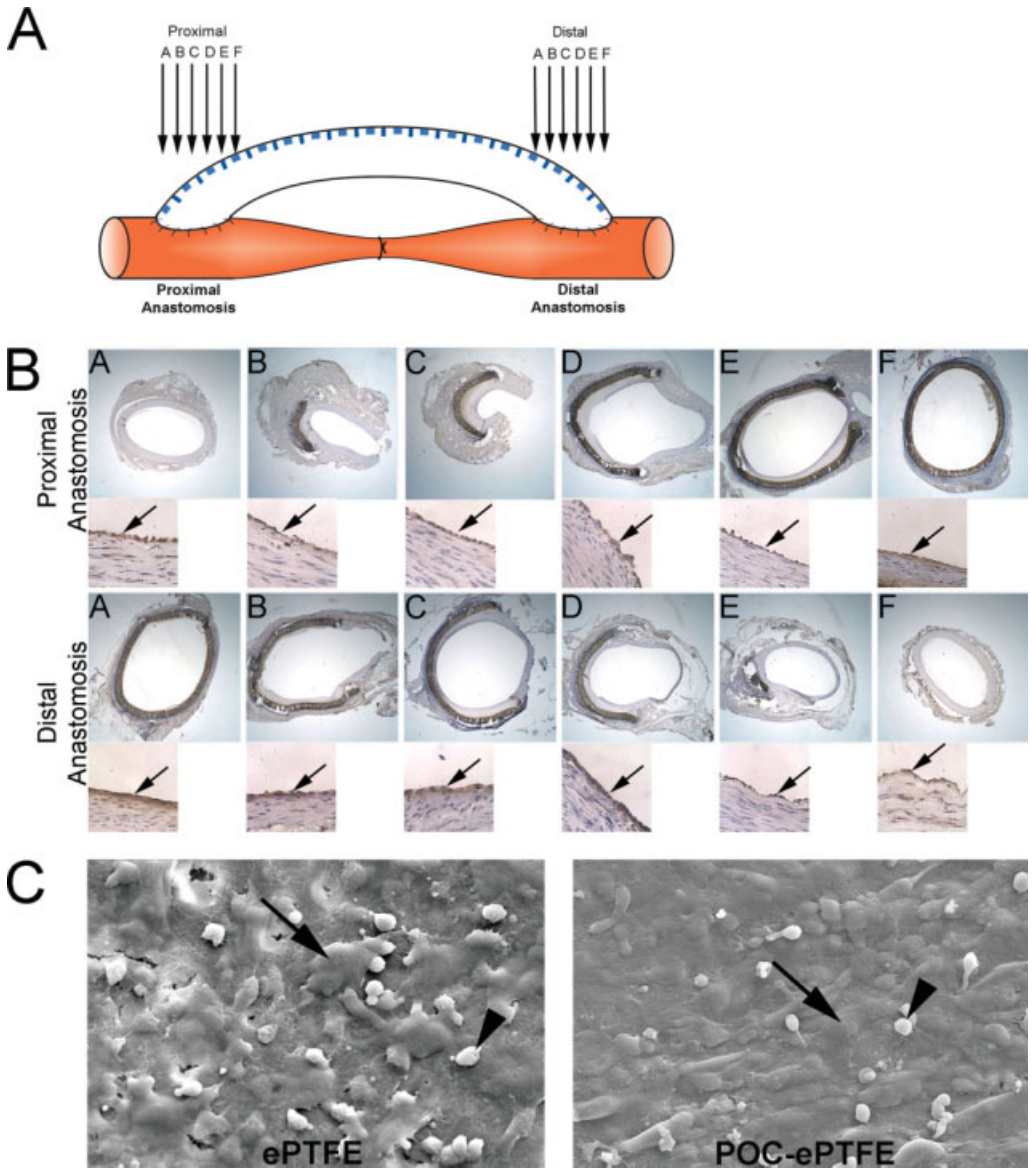


**Figure 4.** POC-ePTFE grafts support endothelialization. A: Schematic demonstrating how the bypass grafts were processed at the proximal and distal anastomoses for assessment of endothelializaton. B: Immunohistochemical assessment of endothelial cell staining progressing from proximal-to-distal (A-to-F) for the proximal and distal anastomoses. Larger picture represents the whole image at 1× magnification. Smaller inset represents the endothelial cell layer at 40× magnification. C: Scanning electron microscopy of the lumen of the control ePTFE and POC-ePTFE bypass grafts demonstrating a continuous endothelial cell monolayer (arrows) on both the control ePTFE and POC-ePTFE grafts. Arrowheads indicate leukocytes. 50 μm scale bar. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Most studies that involve the surface modification of vascular grafts have focused on the potential thrombogenic characteristics of the blood-biomaterial interactions (for review, see[15]). Other than a few studies of drug-eluting grafts,[22,23] there is a dearth of literature on the characterization of thrombogenicity and neointimal hyperplasia of a biomaterial coating for vascular applications. Through a very systematic histological and functional characterization, this study confirms the nonthrombogenic nature of POC and a benign tissue response that did not lead to significant neointimal hyperplasia relative to control uncoated ePTFE grafts, the current standard of care. Blood velocity measurements were within the normal range and significantly below velocity values that are consistent with hemodynamically significant lesions. These findings represent a significant step forward for the intravascular use of this novel biodegradable material in humans, as the coated grafts were not associated with any negative tissue response. The animal model used was based on a previously established porcine model of prosthetic bypass grafting that results in reproducible neointimal hyperplasia.[20]

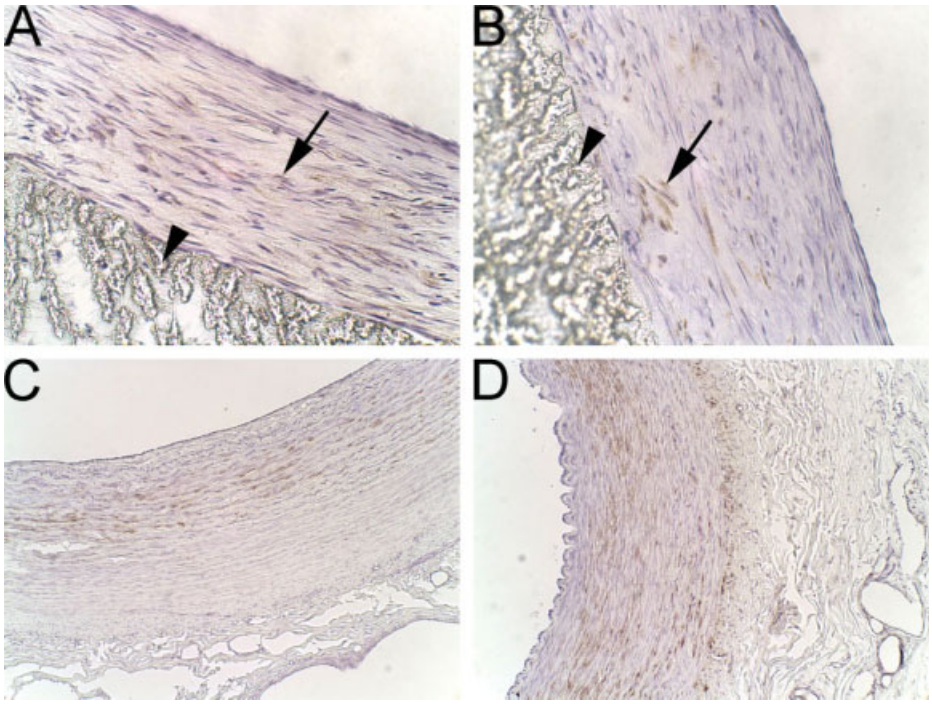


**Figure 5.** POC-ePTFE grafts do not increase leukocyte infiltration. Immunohistochemical analysis for CD45 positive leukocytes (cells stained brown) in control ePTFE grafts (A and C) and POC-ePTFE bypass grafts (B and D). Arrows point to CD45 positive staining leukocytes. Arrowheads denote graft material. (A) and (B) are 40× magnification. (C) and (D) are 10× magnification. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Endothelialization is important for the long-term function of intravascular devices such as stents and vascular grafts.[24,25] In fact, this point has been greatly emphasized with the recent data pertaining to drug-eluting coronary stents. Although early data was encouraging and demonstrated reduced rates of restenosis and late lumen loss, late results of drug-eluting coronary stents have been disappointing.[26] Several pooled or meta-analysis studies have revealed either similar mortality or worse mortality with these drug-eluting stents when compared with bare-metal stents.[27,28] This increased mortality appears to be secondary to an increased rate of in-stent thrombosis.[29] The drug-eluting stents, while inhibiting vascular smooth muscle cell proliferation, are also inhibiting endothelial cell proliferation.[30] As a result, the stent is not covered by endothelial cells and remains thrombogenic. It remains to be determined if the drug or the polymer coating the stent, or both, are inhibiting the growth of endothelial cells. Regardless, the outcome has been disappointing to clinicians. The results from our study confirm that the presence of POC does not inhibit the normal endothelialization response to ePTFE in pigs at the peri-anastomotic sites. Six areas progressing from proximal to distal in each anastomosis were analyzed immunohistochemically for the presence of von Willebrand factor. Although endothelial cells are present, it remains to be determined whether the neoendothelium is functional. A functional endothelium is critical to blood vessel homeostasis, angiogenesis, and overall function.

The inflammatory response to a permanently implanted intravascular device may determine its long-term function.[31–33] We assessed whether POC stimulated or inhibited, relative to ePTFE, the induction of inflammation following prosthetic bypass grafting. Grafts were assessed immunohistochemically for the presence of CD45 positive leukocytes as well as for monocytes and macrophages using the MAC387 antibody. Inflammation and neointimal hyperplasia within the POC-ePTFE grafts was comparable to that of control-uncoated ePTFE grafts. The blood and vascular tissue response to POC is a significant improvement when compared with fibrin-coated ePTFE grafts that were also tested in a porcine animal model.[34] The fact that POC does not enhance the inflammatory response induced by ePTFE suggests that it is a viable delivery platform for drugs designed to inhibit thrombosis and neointimal hyperplasia and/or promote *in vivo* endothelialization for this prosthetic material.

Lastly, our data suggested that POC was still present on the graft samples harvested at 4 weeks, indicating that it had not yet degraded. Depending on the degree of crosslinking, choice of diol, and the inclusion of nanopores, poly(diol citrates) may degrade on a timescale ranging from weeks to

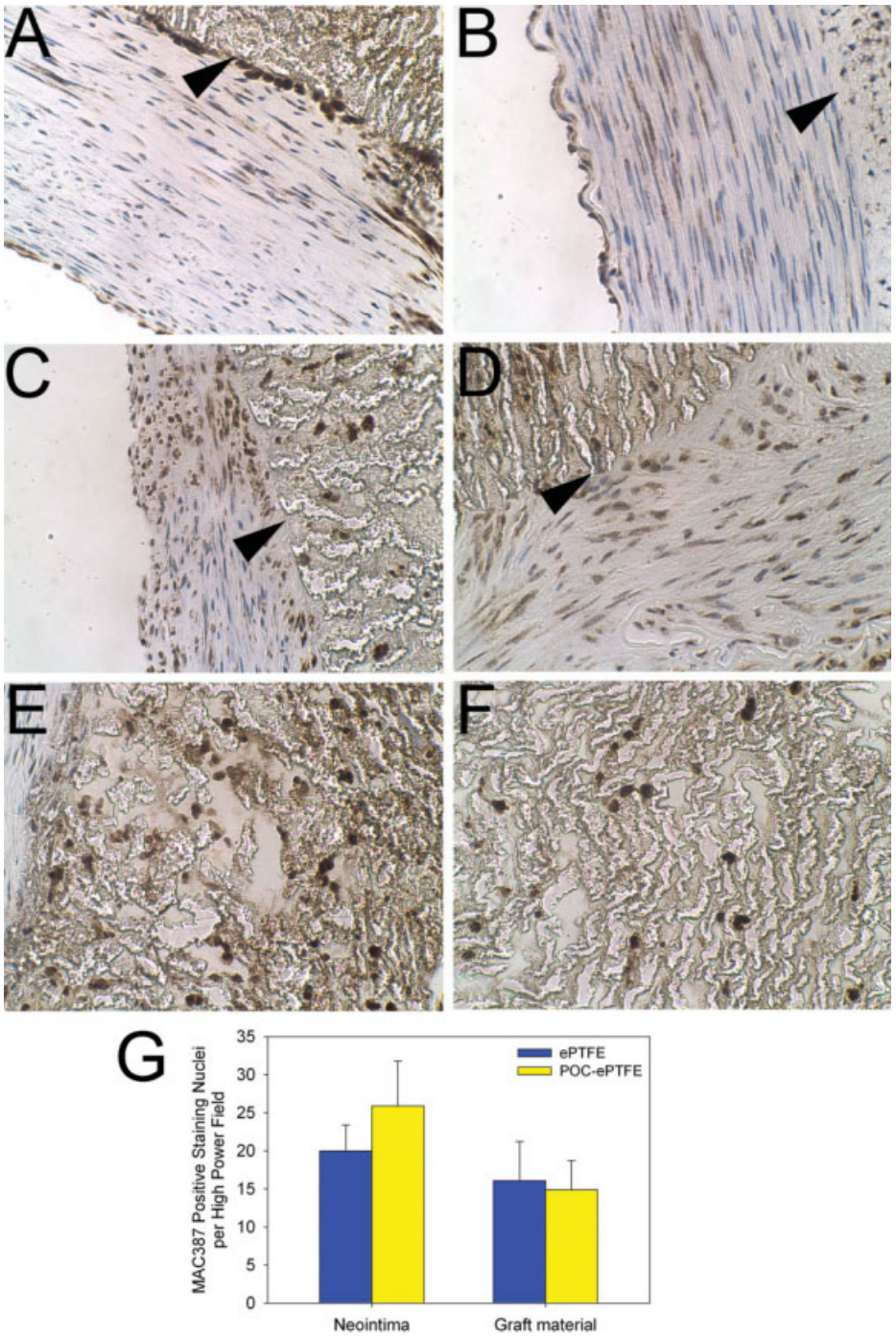


**Figure 6.** POC-ePTFE grafts do not increase monocyte/macrophage infiltration. Immunohistochemical analysis for monocytes/macrophages (cells stained brown) using the MAC387 antibody in control ePTFE grafts (A, C, and E) and POC-ePTFE grafts (B, D, and F). Arrowheads denote interface between graft material and neointimal hyperplasia. Images were obtained with 40× magnification. (G) Objective assessment of MAC387 positive staining cells per high power field in the neointima and in the graft material. The differences between groups were not statistically significant. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

years.[18,35] For the crosslinking conditions used, one would expect a 20–25% degradation based on *in vitro* degradation profiles. It is difficult, if not impossible, to accurately evaluate the extent of POC degradation *in vivo* in this animal model; nevertheless, our data confirm that there are no untoward tissue responses to POC relative to native ePTFE. Over time, one would expect a foreign body response that is in line with that caused by only having ePTFE present at the intervention site.

In conclusion, this study describes the blood and vascular tissue response to poly(diol citrate)-coated

ePTFE vascular grafts. Poly(diol citrates) may offer a powerful platform for the simple modification of ePTFE vascular grafts in order to improve the safety and efficacy of revascularization procedures. By all measures in this study, the poly(diol citrate) coating did not elicit thrombosis, inflammation, or restenosis.

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## References

1. Rosamond W, Flegal K, Friday G, Furie K, Go A, Greenlund K, Haase N, Ho M, Howard V, Kissela B, Kittner S, Lloyd-Jones D, McDermott M, Meigs J, Moy C, Nichol G, O'Donnell CJ, Roger V, Rumsfeld J, Sorlie P, Steinberger J, Thom T, Wasserthiel-Smoller S, Hong Y; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics–2007 update: A report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation 2007;115:e69–e171.
2. Chew DK, Nguyen LL, Owens CD, Conte MS, Whittemore AD, Gravereaux EC, Menard MT, Belkin M. Comparative analysis of autogenous infrainguinal bypass grafts in African Americans and Caucasians: The association of race with graft function and limb salvage. J Vasc Surg 2005;42:695–701.
3. Green RM, Abbott WM, Matsumoto T, Wheeler JR, Miller N, Veith FJ, Money S, Garrett HE. Prosthetic above-knee femoropopliteal bypass grafting: five-year results of a randomized trial. J Vasc Surg 2000;31:417–425.
4. Eagleton MJ, Ouriel K, Shortell C, Green RM. Femoral-infrapopliteal bypass with prosthetic grafts. Surgery 1999;126:759–764.
5. Abbott WM, Megerman J, Hasson JE, Litalien G, Warnock DF. Effect of compliance mismatch on vascular graft patency. J Vasc Surg J Vasc Surg 1987;5:376–382.
6. Clowes AW, Gown AM, Hanson SR, Reidy MA. Mechanisms of arterial graft failure. 1. Role of cellular proliferation in early healing of Ptfe prostheses. Am J Pathol 1985;118:43–54.
7. Clowes AW, Kirkman TR, Clowes MM. Mechanisms of arterial graft failure. II. Chronic endothelial and smooth muscle cell proliferation in healing polytetrafluoroethylene prostheses. J Vasc Surg 1986;3:877–884.
8. Loth F, Jones SA, Zarins CK, Giddens DP, Nassar RF, Glagov S, Bassiouny HS. Relative contribution of wall shear stress and injury in experimental intimal thickening at PTFE end-to-side arterial anastomoses. J Biomech Eng 2002;124:44–51.
9. Kidane AG, Salacinski H, Tiwari A, Bruckdorfer KR, Seifalian AM. Anticoagulant and antiplatelet agents: Their clinical and device application(s) together with usages to engineer surfaces. Biomacromolecules 2004;5:798–813.
10. Ascer E, Gennaro M, Pollina RM, Ivanov M, Yorkovich WR, Ivanov M, Lorensen E. Complementary distal arteriovenous fistula and deep vein interposition: A five-year experience with a new technique to improve infrapopliteal prosthetic bypass patency. J Vasc Surg 1996;24:134–143.
11. Batson RC, Sottiurai VS, Craighead CC. Linton patch angioplasty. An adjunct to distal bypass with polytetrafluoroethylene grafts. Ann Surg 1984;199:684–693.
12. Griffiths GD, Nagy J, Black D, Stonebridge PA. Randomized clinical trial of distal anastomotic interposition vein cuff in infrainguinal polytetrafluoroethylene bypass grafting. Br J Surg 2004;91:560–562.
13. Stonebridge PA, Prescott RJ, Ruckley CV. Randomized trial comparing infrainguinal polytetrafluoroethylene bypass grafting with and without vein interposition cuff at the distal anastomosis. The Joint Vascular Research Group. J Vasc Surg 1997;26:543–550.
14. Taylor RS, Loh A, Mcfarland RJ, Cox M, Chester JF. Improved Technique for Polytetrafluoroethylene Bypass-Grafting—Long-Term Results Using Anastomotic Vein Patches. Br J Surg 1992;79:348–354.
15. Kapadia MR, Popowich DA, Kibbe MR. Modified prosthetic vascular conduits. Circulation 2008;117:1873–1882.
16. Yang J, Motlagh D, Allen JB, Webb AR, Kibbe MR, Aalami O, Kapadia M, Carroll TJ, Ameer GA. Modulating expanded polytetrafluoroethylene vascular graft host response via citric acid-based biodegradable elastomers. Adv Mater 2006; 18:1493–1498.
17. Yang J, Webb AR, Ameer GA. Novel citric acid-based biodegradable elastomers for tissue engineering. Adv Mater 2004;16:511–516.
18. Yang J, Webb AR, Pickerill SJ, Hageman G, Ameer GA. Synthesis and evaluation of poly(diol citrate) biodegradable elastomers. Biomaterials 2006;27:1889–1898.
19. Motlagh D, Allen J, Hoshi R, Yang J, Lui K, Ameer G. Hemocompatibility evaluation of poly(diol citrate) in vitro for vascular tissue engineering. J Biomed Mater Res A 2007;82:907–916.
20. Kapadia MR, Aalami OO, Najjar SF, Jiang Q, Murar J, Lyle B, Eng JW, Kane B, Carroll T, Cahill PM, Kibbe MR. A reproducible porcine ePTFE arterial bypass model for neointimal hyperplasia. J Surg Res 2008;148:230–237.
21. Allen J, Khan S, Serrano MC, Ameer G. Characterization of porcine circulating progenitor cells: Toward a functional endothelium. Tissue Eng Part A 2008;14:183–194.
22. Cagiannos C, Abul-Khoudoud OR, DeRijk W, Shell DH, Jennings LK, Tolley EA, Handorf CR, Fabian TC. Rapamycin-coated expanded polytetrafluoroethylene bypass grafts exhibit decreased anastomotic neointimal hyperplasia in a porcine model. J Vasc Surg 2005;42:980–988.
23. Lee BH, Nam HY, Kwon T, Kim SJ, Kwon GY, Jeon HJ, Lim HJLWK, Park JS, Ko JY, Kim DJ. Paclitaxel-coated expanded polytetrafluoroethylene haemodialysis grafts inhibit neointimal hyperplasia in porcine model of graft stenosis. Nephrol Dial Transplant 2006;21:2432–2438.
24. Zilla P. Endothelialization of vascular grafts. Curr Opin Cardiol 1991;6:877–886.
25. Zilla P, Fasol R, Deutsch M, Fischlein T, Minar E, Hammerle A, Krupicka O, Kadletz M. Endothelial-cell seeding of polytetrafluoroethylene vascular grafts in humans—a preliminary-report. J Vasc Surg 1987;6:535–541.
26. Iakovou I, Schmidt T, Bonizzoni E, Ge L, Sangiorgi GM, Stankovic G, Airoldi F, Chieffo A, Montorfano M, Carlino M, et al. Incidence, predictors, and outcome of thrombosis after successful implantation of drug-eluting stents. JAMA 2005;293:2126–2130.
27. Lagerqvist B, James SK, Stenestrand U, Lindback J, Nilsson T, Wallentin L. Long-term outcomes with drug-eluting stents versus bare-metal stents in Sweden. N Engl J Med 2007; 356:1009–1019.
28. Spaulding C, Daemen J, Boersma E, Cutlip DE, Serruys PW. A pooled analysis of data comparing sirolimus-eluting stents with bare-metal stents. N Engl J Med 2007;356:989–997.

29. Maisel WH. Unanswered questions–drug-eluting stents and the risk of late thrombosis. N Engl J Med 2007;356:981–984.
30. Wernick MH, Jeremias A, Carrozza JP. Drug-eluting stents and stent thrombosis: A cause for concern? Coronary Artery Dis 2006;17:661–665.
31. Murray-Wijelath J, Lyman DJ, Wijelath ES. Vascular graft healing. III. FTIR analysis of ePTFE graft samples from implanted bigrafts. J Biomed Mater Res B Appl Biomater 2004;70B:223–232.
32. Shindo S, Motohashi S, Katsu M, Kaga S, Inoue H, Matsumoto M. Coated prostheses are associated with prolonged inflammation in aortic surgery: A cost analysis. Artif Organs 2008;32:183–187.
33. Zippel R, Wilhelm L, Hoene A, Walschus U, Ueberrueck T, Schlosser M. Local tissue reaction and differentiation of the prosthesis-specific antibody response following functional implantation of vascular grafts in pigs. J Biomed Mater Res B Appl Biomater 2008;85B:334–342.
34. Walpoth BH, Zammaretti P, Cikirikcioglu M, Khabiri E, Djebaili MK, Pache JC, Tille JC, Aggoun Y, Morel D, Kalangos A, Hubbell JA, Zisch AH. Enhanced intimal thickening of expanded polytetrafluoroethylene grafts coated with fibrin or fibrin-releasing vascular endothelial growth factor in the pig carotid artery interposition model. J Thorac Cardiovasc Surg 2007;133:1163–1170.
35. Hoshi RA, Behl S, Ameer GA. Biomedical materials: Nanoporous biodegradable elastomers. Adv Mater 2009; 21:188–192.