Modulating Expanded Polytetrafluoroethylene Vascular Graft Host Response via Citric Acid-Based Biodegradable Elastomers**

By *Jian Yang, Delara Motlagh, Josephine B. Allen, Antonio R. Webb, Melina R. Kibbe, Oliver Aalami, Muneera Kapadia, Timothy J. Carroll*, and *Guillermo A. Ameer**

Atherosclerotic vascular disease, in the form of coronary artery and peripheral vascular disease, remains the leading cause of mortality in the United States.[1] For many patients, suitable vein autografts are not available.[2] Synthetic grafts made from Dacron (polyethylene terephthalate) or expanded polytetrafluoroethylene (ePTFE) are mostly used in large-diameter (>6 mm inner diameter) blood-vessel applications. However, their use in small-diameter blood vessels has been fraught with poor patency due to early graft occlusion from thrombosis.[3,4] Surface-modification protocols mostly target ePTFE vascular grafts, the current standard-of-care, as their innate surface is thrombogenic and their intrinsic hydrophobicity can limit endothelium formation.[3,5–8] Modifications to improve the thromboresistance of the graft have focused on the coating or immobilization of biomacromolecules or nondegradable polymers to promote endothelialization of the graft's lumen.[6,9–19] However, concerns regarding changes to the graft's compliance, host responses to the coating material, transmission of pathogens, high costs, and long-term patency still remain.[3,20] To date, no one has investigated the use of synthetic biodegradable polymers as a means to tissue-engineer a functional endothelium on ePTFE grafts. We demonstrate that the biodegradable elastomer poly(1,8-octanediol citrate) (POC) can confer improved biocompatibility characteristics on ePTFE vascular grafts without affecting graft compliance. The approach ultimately aims to improve early thromboresistance and perhaps inhibit neointimal hyperplasia by promoting POC-mediated mechanical interlocking between a newly formed endothelial layer and the underlying ePTFE fibrils and nodes.

The POC synthesis and interfacial deposition methods presented herein are convenient and inexpensive, factors that are important to the widespread use and industrial scale-up for clinical application. POC prepolymer was synthesized via the polycondensation of citric acid and 1,8-octanediol as previously described[21] and dissolved in ethanol for further use with the ePTFE grafts. Unlike other polycondensation reactions, which require high temperatures (normally, >200 °C), catalysts or enzymes, and high vacuum,[22–24] synthesis of POC can be conducted under relatively low temperatures (typically 60–80 °C, or as low as 37 °C, if necessary) without vacuum and catalysts.[21,25] The time to achieve total degradation of POC, which may range from a few months to over a year, can be controlled with the degree of crosslinking via the extent of reaction, choice of diol, molar ratio of the diol to citric acid, and doping with hydrophilic crosslinking monomers such as *N*-methyldiethanolamine and glycerol.[25] POC's modulus and elongation-at-break range from 0.5–11 MPa and 100–400 %, respectively, depending on the synthesis conditions used.[25] A spin-shearing technique was developed to evenly coat the POC prepolymer onto the luminal surface of the ePTFE vascular grafts, effectively functionalizing the surface with carboxyl and hydroxyl functional groups. The POC-coated graft was post-polymerized at 80 °C for two days to create an elastomeric biodegradable polymer network on the ePTFE. The latter procedure is referred to as interfacial in situ polycondensation.

POC modification of ePTFE (POC–ePTFE) significantly changed the surface energy of the ePTFE grafts without affecting graft compliance. The lumen of the graft was mostly covered with POC, >95 % as assessed using scanning electron microscopy (SEM) and image-analysis software. Notably, the overall microarchitecture of the fibril and node network of ePTFE is preserved within the deposited POC layer (Fig. 1B). The compliance of POC–ePTFE grafts was not significantly different from that of control ePTFE grafts (Fig. 1C). This finding is due, in part, to the elastomeric nature of POC, which allows it to mobilize according to the expansion or contraction of the PTFE fibrils and nodes when the graft is exposed to pulsatile flow conditions. Surface Fourier transform infrared (FTIR) spectroscopy, X-ray photoelectron spectroscopy (XPS), and contact-angle measurements further confirmed the morphological evidence for successful coating of the ePTFE graft (Fig. 1D–F). From the FTIR data, the broad peaks centered at 3475 and 3215 cm^{-1} were assigned to the hydroxyl-group stretching vibration and ν_{O-H} of the carboxyl groups of POC. The peaks at 2925 and 2850 cm^{-1} and the peaks within the range 1690–1750 cm^{-1} in spectrum B shown in Figure 1D were assigned to the –CH$_2$– and carbonyl (C=O) groups of POC, respectively. XPS analysis (Fig. 1E)

[*] Prof. G. A. Ameer, Dr. J. Yang, Dr. D. Motlagh, J. B. Allen,
A. R. Webb, Dr. T. J. Carroll
Biomedical Engineering Department, Northwestern University
2145 Sheridan Road, Evanston, IL 60208 (USA)
E-mail: g-ameer@northwestern.edu
Dr. M. R. Kibbe, Dr. O. Aalami, Dr. M. Kapadia
Division of Vascular Surgery
Northwestern University Feinberg School of Medicine
303 East Chicago Avenue, Chicago, IL 60611 (USA)
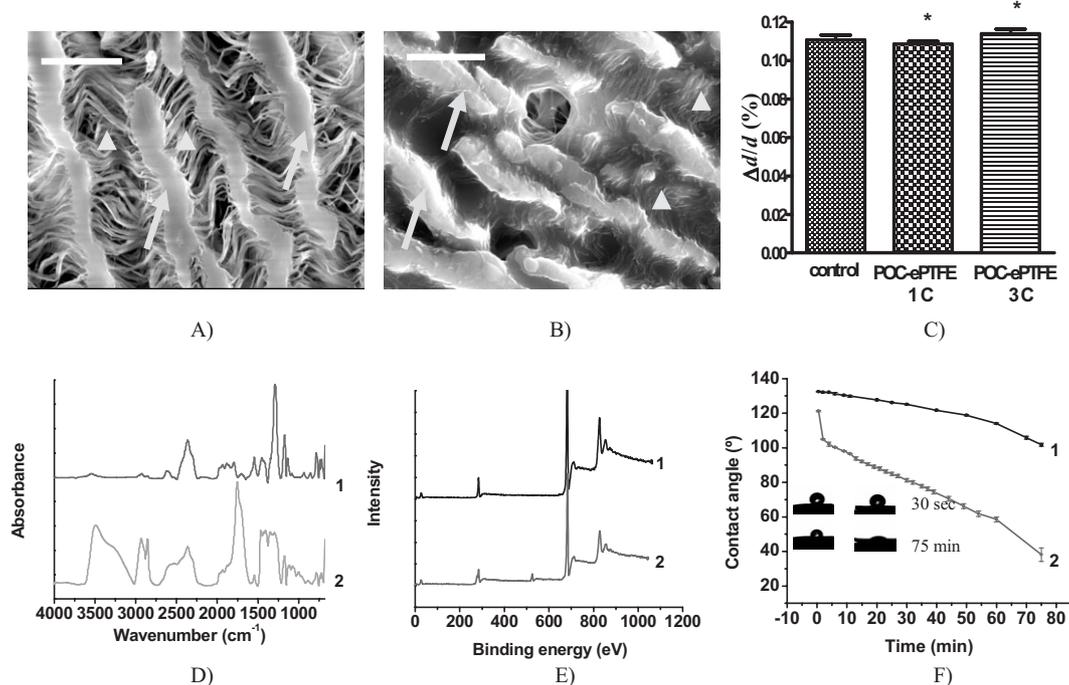
Figure 1. Characterization of POC–ePTFE grafts. A) SEM image of a control ePTFE graft. B) SEM image of a POC–ePTFE graft with three POC coatings. Scale bars in (A) and (B) are 20 μm; arrows show nodes and arrowheads indicate fibrils. C) Effect of the POC coating on the compliance of the ePTFE graft. Compliance of the control ePTFE grafts and POC–ePTFE grafts with one coating (1 C) and three coatings (3 C). * indicates that $P > 0.05$ versus the control (data were expressed as mean ± standard error of the mean, $N = 60$). D) Surface FTIR analysis of treated and untreated grafts: 1) control ePTFE and 2) ePTFE with three coatings of POC. E) XPS analysis of the grafts: 1) control ePTFE, and 2) POC–ePTFE with three coatings. F) Effect of POC coating on the water-in-air contact angle: 1) untreated ePTFE, and 2) POC–ePTFE graft with three coatings.

revealed a new peak at approximately 539 eV, which was assigned to O 1s from POC. The fluorine/carbon ratio (F/C) decreased from 2.1 for an unmodified graft[6] to 1.36 for the POC-modified graft. The decrease of F/C was accompanied by an increase of the oxygen/carbon ratio (O/C) from 0 to 0.13. Deposition of POC onto the ePTFE fibril network of the grafts resulted in an equilibrium water-in-air contact angle of $38.1 \pm 3.9°$, which was significantly lower than that of control grafts ($101.7 \pm 0.8°$) (Fig. 1F). The lower contact angle confirms the increased wettability of the graft, which was expected to improve cell-adhesion properties.[16]

POC modified ePTFE has reduced thrombogenicity and enhanced endothelial cell compatibility relative to control ePTFE grafts in vitro. POC–ePTFE significantly inhibited platelet adhesion and aggregation (Fig. 2A–C) in vitro. The adhering platelets on POC–ePTFE could maintain their shape, while the platelets adhered to ePTFE underwent drastic morphology changes and pseudopod formation (Fig. 2B,C), both signs of platelet activation.[26] According to plasma recalcification results, POC significantly delayed the clotting of plasma when compared to tissue-culture polystyrene (TCP) and poly[(L-lactide)-co-glycolide](85/15) (PLGA85/15), a biodegradable polymer that is commonly used in tissue-engineering applications (Fig. 2D). The reasons for the observed POC hemocompatibility data are complex,

with one possibility involving the citric acid moiety of POC. Sodium citrate, a salt form of citric acid, is often used in hospitals as an anticoagulant and the calcium-chelating properties that allow its use as an anticoagulant may be implicated in the observed reduced thrombogenicity of POC. Nevertheless, these data confirmed the favorable thrombogenic properties of POC, and suggest that it may be useful as a coating to improve the hemocompatibility of materials that must come in contact with blood. Regarding the source of autologous cells, recent studies have shown the existence of mature and immature endothelial-like cells circulating in peripheral blood in several species.[27–30] We isolated vascular progenitor cells from pig blood and successfully differentiated them into porcine endothelial-like cells (PE-like cells). The endothelial phenotype of the differentiated progenitor cells was confirmed with positive immunohistochemical staining for von Willebrand factor (vWF) and vascular endothelial-cadherin (VE-cadherin) (Fig. 2E,F). Cells were also positive for functional tests such as acetylated low-density lipoprotein uptake, and baseline or induced nitric oxide and prostacyclin secretion (data not shown). PE-like cells attached, proliferated, and became confluent on POC–ePTFE grafts within ten days (Fig. 2H). In contrast, ePTFE grafts exhibited a random distribution of cell clusters or patches with significant exposure of the underlying ePTFE fibril and node network (Fig. 2G).
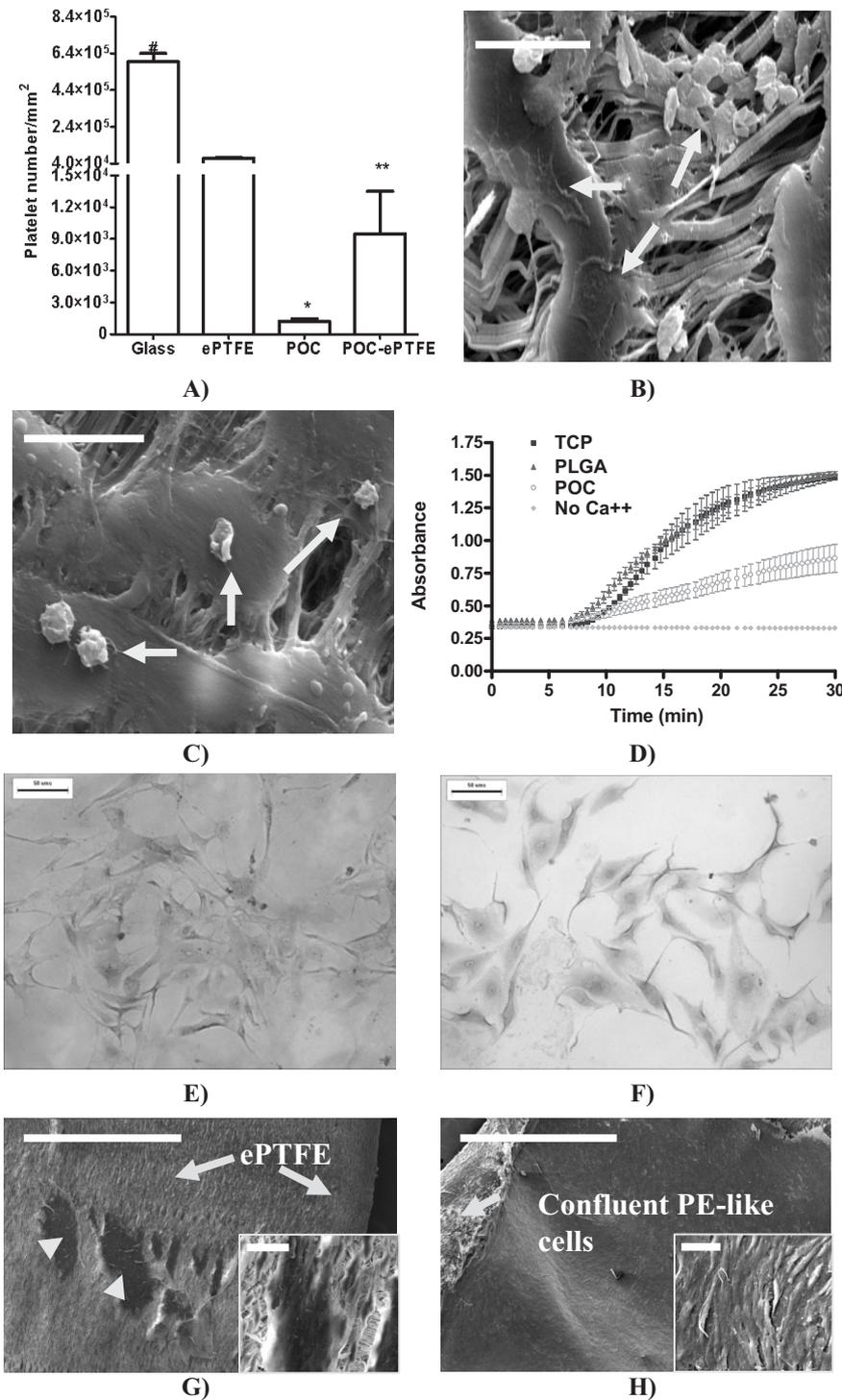
Figure 2. In vitro biocompatibility evaluation of POC–ePTFE grafts. A) Platelet adhesion on ePTFE, POC, POC–ePTFE, and glass. *, **, and # indicate $P < 0.05$ versus ePTFE. B) SEM image of platelets (arrow) on ePTFE. C) SEM image of platelets (arrow) on POC–ePTFE. D) Plasma recalcification clotting profiles. Platelet-poor plasma on TCP (blue), PLGA85/15 (red), and POC (green). ePTFE samples could not be evaluated using this technique due to the opaque nature of the samples. Data were expressed as mean + standard deviation, $N = 6$. E) Immunostaining of PE-like cells on POC for vWF. F) Immunostaining of PE-like cells on POC for VE-cadherin. G) SEM image of PE-like cells on a ePTFE graft. Arrows and arrowheads show the native ePTFE and patches of cells, respectively. H) SEM image of confluent PE-like cells on a POC–ePTFE graft. Arrow shows the cross section of the graft wall. Scale bars in B,C) are 10 μm, E,F) are 50 μm, and G,H) are 1 mm. Inserts in G,H) are close-up views of the graft surface (scale bar is 50 μm).

These data suggest that POC–ePTFE could potentially facilitate complete endothelialization of the graft.

POC modification can significantly improve the hemocompatibility and acute inflammatory response of ePTFE grafts implanted in a porcine iliac artery ePTFE bypass model (Fig. 3A). The patency of the grafts was assessed using magnetic resonance (MR) angiography and histology after one week of implantation (Fig. 3B). Patency of the POC–ePTFE and ePTFE grafts was equal (5:5). Histomorphometric analysis of immunostaining for macrophages using the MAC387 antibody suggested reduced macrophage recruitment activity to the POC–ePTFE graft relative to ePTFE (Fig. 3C,D,G). The thickness of the fibrin coagulum and the deposition of platelet and blood cells within the lumen of the grafts were significantly reduced in the POC–ePTFE grafts (Fig. 3E,F,H). After one week in vivo, the POC coating could still be observed on the grafts, confirming its stability to blood-flow conditions that would be comparable to those found in a human (Fig. 3E).

Currently, there are no synthetic vascular grafts available that exhibit long-term patency for the replacement of small-diameter blood vessels.[5,31,32] In the present study, we used a biodegradable polyester elastomer that is based on citric acid to modify the lumen of ePTFE grafts via a simple spin-shearing method followed by interfacial in situ polycondensation. We demonstrated successful surface modification of ePTFE grafts without affecting graft compliance. The POC interface conferred hydrophilicity, reduced thrombogenicity, facilitated graft endothelialization in vitro, and reduced in vivo macrophage infiltration into the ePTFE grafts. The materials-based approach described in this study represents a paradigm shift and a new starting point towards engineering a functional vascular graft for the replacement of small-diameter blood vessels. Future studies will evaluate whether a tissue-engineered endothelium using a POC–ePTFE graft reduces clotting incidents and improves long-term patency in vivo.
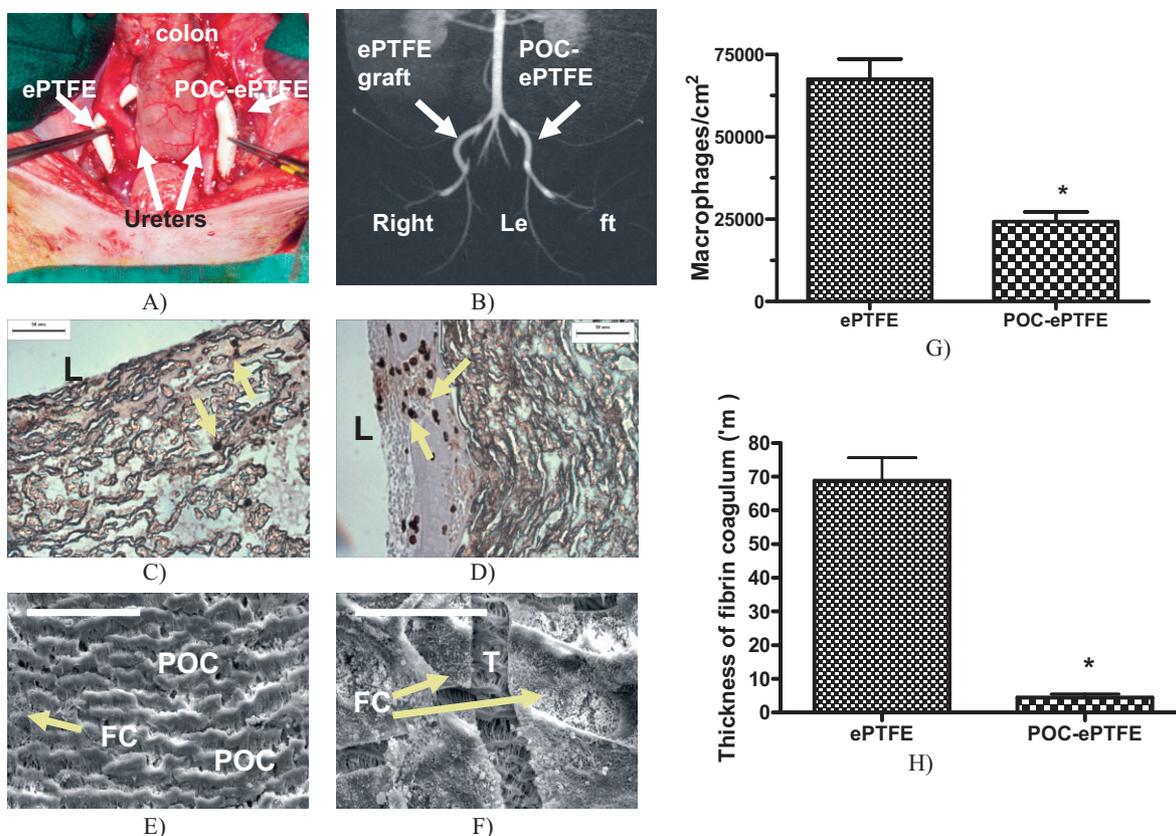
Figure 3. In vivo assessment in pigs of the foreign-body reaction to the grafts. A) Intra-operative photo of the implanted grafts. B) Contrast-enhanced MR angiogram visualizing patency of both ePTFE and POC–ePTFE grafts at seven days. C) MAC 387 stain for macrophages (arrow) on a POC–ePTFE graft (L = lumen, scale bar is 50 μm). D) MAC 387 stain for macrophages (arrows) on a control ePTFE graft (scale bar is 50 μm). E,F) SEM of the lumen of the POC–ePTFE and ePTFE grafts after one week of implantation (FC = fibrin coagulum, T = ePTFE, scale bars are 100 μm). G) Macrophage density on both ePTFE and POC–ePTFE grafts; * indicates $P < 0.05$ versus ePTFE. H) Thickness of fibrin coagulum on both ePTFE and POC–ePTFE grafts; * indicates $P < 0.05$ versus ePTFE. For macrophage density and thickness of fibrin coagulum measurements, data were expressed as mean ± standard error of the mean, $N = 60$.

Experimental

Polymer Synthesis: The synthesis and characterization of POC was previously published [21,25]. 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 standard-wall nonstretch ePTFE grafts (Gore-Tex, W. L. Gore & Associates, Flagstaff, AZ, 6 mm inner diameter) was modified by mechanically coating a POC layer interlocked with the nodes and fibrils of ePTFE through a spin-shearing method. 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 a 6 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 two days to obtain POC–ePTFE grafts.

Characterization of the POC–ePTFE Graft: POC–ePTFE grafts and unmodified ePTFE grafts (control) were cut open into small pieces (1 cm × 2 cm). The sample with the modified side up was examined by diffuse reflectance infrared Fourier transform spectroscopy (Thermo Nicolet Nexus 870, Keck II, NUANCE, Northwestern University, Evaston, IL), XPS (Keck II, NUANCE, Northwestern University, Evaston, IL), water-in-air contact-angle measurements using a Ramé-Hart goniometer and an imaging system (Ramé-Hart, Mouttain Lake, NJ), and SEM (Hitachi 3500N, EPIC, Northwestern University, Evaston, IL). The coverage of the POC coating was calculated by measuring the POC-covered area and uncovered area by analyzing SEM pictures using image analysis software (Image-Pro Plus V.4.0, MediaCybernetics, Silver Spring, MD). Compliance ($\Delta d/d$ %, d is the outer diameter of the graft) was defined as the change in diameter of the grafts during the rise in pressure between diastole and systole (typically from 80 to 120 mm Hg), and was expressed as a percentage. Measurements were conducted on a custom-made in vitro closed-loop flow system. Computer-controlled pressurization or a pulsatile blood pump (Harvard Apparatus, Hollister, MA) was used to control pulsation frequency and pressure ranges. A light-emitting diode micrometer (Keyence LS7000, Higashi-Yodogawa, Osaka) was used to non-invasively measure radial distension. The pressure and distension data

over time was recorded with LabVIEW software (National Instruments, Austin, TX). Three POC–ePTFE and three ePTFE grafts (5 cm in length) were used for compliance measurements. Twenty tests were performed on each graft.

In vitro Hemocompatibility Evaluation of POC–ePTFE: The methods used to collect and prepare the platelets used in this study have been approved by the Institutional Review Board and the Office for the Protection of Research Subjects at Northwestern University. Blood was drawn from healthy adult volunteers by venipuncture into acid citrate dextrose anticoagulant (solution A; BD Franklin Lakes, NJ). Platelet-rich plasma (PRP) was prepared as previously described [26]. POC–ePTFE, ePTFE, POC, and glass samples were incubated with 100 μL of PRP (5.4×10^8 mL^{-1}) for 1 h at 37 °C under static conditions. The number of adherent platelets was determined by detecting the amount of lactate dehydrogenase using a modification of the methods described by Tamada et al. [33]. The morphology of the adherent platelets was evaluated using SEM. Clotting times on POC were determined using a plasma recalcification assay. Control surfaces consisted of TCP and PLGA85/15 (Aldrich, Milwaukee, WI) [34]. Due to the opaque nature of POC–ePTFE and ePTFE they were not evaluated with this method.

Seeding of PE-like Cells on POC–ePTFE Grafts: Mononuclear cells were isolated from the peripheral blood of domestic pigs using a Histopaque (Sigma-Aldrich, St. Luios, MO) density-gradient centrifugation method [35]. Cells were seeded onto fibronectin-coated plates (BD Biosciences, Bedford, MA), and cultured in endothelial cell basal medium-2 (Clonetics; Baltimore, MD) supplemented with 17 % fetal bovine serum, human fibroblast growth factor, vascular endothelial growth factor, human recombinant insulin-like growth factor (R3-IGF-1), ascorbic acid, gentamicin, amphotercin, and heparin. Adherent cells were cultured for two weeks. Prior to cell seeding, the cells were grown on POC films and stained with vWF (DakoCytomation, Carpenteria, CA) and VE-cadherin (Sigma Aldrich, Milwaukee, WI) to confirm their endothelial phenotype. POC–ePTFE and unmodified grafts (6 cm in length and 6 mm in diameter) were gas-sterilized via exposure to ethylene oxide and placed in a culture dish (150 mm × 15 mm, Becton Dickinson, NY). A 1.8 mL suspension of cells (3.9×10^6 cells mL^{-1}) was injected into the lumen of the grafts and incubated at 37 °C for 60 min. The graft was rotated 180°, and an additional 1.8 mL suspension of cells was injected into the lumen of the grafts. The cell suspension was incubated in the grafts for 60 min prior to adding 80 mL of fresh culture medium to each culture dish. After culture for ten days, the grafts were cut into three 2 cm long segments. Each segment was subsequently cut open and fixed with 2.5 % glutaraldehyde in phosphate-buffered saline for observation using SEM.

Iliac Artery Bypass Model in Pigs: All procedures and animal care were performed in accordance with the regulations of the Northwestern University Animal Care and Use Committee (NU-ACUC). Five male pigs (Yorkshire Landrace, Oak Hill Genetics, Fanning Farms) weighing 20 to 25 kg were used in the study. Bilateral common iliac artery bypass grafting was performed with a POC–ePTFE graft on one side and a control graft on the contralateral side. Animals were monitored for the patency of the grafts via MRI at the Center for Advanced MRI, Northwestern University.

Histology and Immunohistochemistry Evaluation of the Grafts: The grafts and adjacent 3 cm segments of attached vessel at each anastomosis were harvested and cut into two parts from the middle. A 1 cm long graft from each part was fixed in a 2.5 % glutaraldehyde solution for morphological analysis using SEM. The rest of the grafts with anastomosis were fixed in 10 % neutral buffered formalin (Sigma, Milwaukee, WI). Formalin-fixed grafts were embedded in paraffin and sectioned into five equal segments from the proximal and distal anastomoses. Macrophage staining was performed with MAC387 antibody (Serotec, UK) on 5 μm thick sections. The sections were imaged with a Nikon microscope (Nikon Eclipse, TE2000-U) equipped with a photometrics CoolSNAP HQ (Silver Spring, MD) at 400× magnification. To quantify the macrophage density and the thickness of fibrin coagulum, six images were randomly acquired from each ring-shaped section. Since macrophages appeared mostly at the lumen/graft interface, the interface was taken as the reference plus 250 μm into the graft's wall. MAC387 positive cells were divided by the area of interest, which included part of the graft's wall area and neighboring fibrin coagulum area if applicable. The thickness of fibrin coagulum was analyzed using the image-analysis software. Sections from five pigs were analyzed and averaged. All the evaluations were analyzed without knowing the identity of the sections in each slide in advance.

Statistical Analysis: Platelet adhesion and plasma recalcification data were analyzed using GraphPad Prism (4.00) with one-way analysis of variance and Newman-Keuls multiple comparison test. The macrophage density and thickness of fibrin coagulum were analyzed using a two-tail Student's *t*-test. Data were taken to be significant when a *P* value of 0.05 or less was obtained.

[1] T. Thom, N. Haase, W. Rosamond, V. J. Howard, J. Rumsfeld, T. Manolio, Z.-J. Zheng, K. Flegal, C. O'Donnell, S. Kittner, D. Lloyd-Jones, D. C. Goff, Jr, Y. Hong, R. Adams, G. Friday, K. Furie, P. Gorelick, B. Kissela, J. Marler, J. Meigs, V. Roger, S. Sidney, P. Sorlie, J. Steinberger, S. Wasserthiel-Smoller, M. Wilson, P. Wolf, *Circulation* **2006**, *113*, e85.

[2] A. Tiwari, H. Salacinski, A. M. Seifalian, G. Hamilton, *Cardiovasc. Surg.* **2002**, *10*, 191.

[3] L. Xue, H. P. Greisler, *J. Vasc. Surg.* **2003**, *37*, 472.

[4] M. J. Eagleton, K. Ouriel, C. Shortell, R. M. Green, *Surgery* **1999**, *126*, 759.

[5] R. Y. Kannan, H. J. Salacinski, P. E. Butler, G. Hamilton, A. M. Seifalian, *J. Biomed. Mater. Res.* **2005**, *74*, 570.

[6] I. Noh, S. L. Goodman, J. A. Hubbell, *J. Biomater. Sci. Polym. Ed.* **1998**, *9*, 407.

[7] A. Kusaba, C. R. Fischer, T. J. Matulewski, T. Matsumoto, *Am. Surg.* **1981**, *47*, 347.

[8] M. B. Herring, R. Dilley, R. A. Jersild, Jr., L. Boxer, A. Gardner, J. Glover, *Ann. Surg.* **1979**, *190*, 84.

[9] H. J. Salacinski, A. Tiwari, G. Hamilton, A. M. Seifalian, *Med. Biol. Eng. Comput.* **2001**, *39*, 609.

[10] S. A. Parikh, E. R. Edelman, *Adv. Drug Delivery Rev.* **2000**, *42*, 139.

[11] J. Li, M. J. Singh, P. R. Nelson, G. M. Hendricks, M. Itani, M. J. Rohrer, B. S. Cutler, *J. Surg. Res.* **2002**, *105*, 200.

[12] P. H. Lin, C. Chen, R. L. Bush, Q. Yao, A. B. Lumsden, S. R. Hanson, *J. Vasc. Surg.* **2004**, *39*, 1322.

[13] J. Laredo, L. Xue, V. A. Husak, J. Ellinger, H. P. Greisler, *Am. J. Surg.* **2003**, *186*, 556.

[14] H. W. Jun, J. L. West, *J. Biomed. Mater. Res.* **2005**, *72*, 131.

[15] C. R. Wang, Q. Zhang, S. Uchida, M. Kodama, *J. Biomed. Mater. Res.* **2002**, *62*, 315.

[16] J. Yang, Y. Wan, J. Yang, J. Bei, S. Wang, *J. Biomed. Mater. Res.* **2003**, *67*, 1139.

[17] Q. Zhang, C. Wang, Y. Babukutty, T. Ohyama, M. Kogoma, M. Kodama, *J. Biomed. Mater. Res.* **2002**, *60*, 502.

[18] D. Y. Tseng, E. R. Edelman, *J. Biomed. Mater. Res.* **1998**, *42*, 188.

[19] R. Sipehia, M. Liszkowski, A. Lu, *J. Cardiovasc. Surg.* **2001**, *42*, 537.

[20] R. M. Nerem, D. Seliktar, *Annu. Rev. Biomed. Eng.* **2001**, *3*, 225.

[21] J. Yang, A. R. Webb, G. A. Ameer, *Adv. Mater.* **2004**, *16*, 511.

[22] A. Mahapatro, A. Kumar, B. Kalra, R. A. Gross, *Macromolecules* **2004**, *37*, 35.

[23] A. Takasu, Y. Iio, Y. Oishi, Y. Narukawa, T. Hirabayashi, *Macromolecules* **2005**, *38*, 1048.

[24] Y. D. Wang, G. A. Ameer, B. J. Sheppard, R. Langer, *Nat. Biotechnol.* **2002**, *20*, 602.

[25] J. Yang, A. R. Webb, S. J. Pickerill, G. Hageman, G. A. Ameer, *Biomaterials* **2006**, *27*, 1889.

[26] J. M. Grunkemeier, W. B. Tsai, T. A. Horbett, *J. Biomed. Mater. Res.* **1998**, *41*, 657.

[27] T. Asahara, T. Murohara, A. Sullivan, M. Silver, R. van der Zee, T. Li, B. Witzenbichler, G. Schatteman, J. M. Isner, *Science* **1997**, *275*, 964.

[28] Q. Shi, S. Rafii, M. H. Wu, E. S. Wijelath, C. Yu, A. Ishida, Y. Fujita, S. Kothari, R. Mohle, L. R. Sauvage, M. A. Moore, R. F. Storb, W. P. Hammond, *Blood* **1998**, *92*, 362.

[29] M. Peichev, A. J. Naiyer, D. Pereira, Z. Zhu, W. J. Lane, M. Williams, M. C. Oz, D. J. Hicklin, L. Witte, M. A. Moore, S. Rafii, *Blood* **2000**, *95*, 952.

[30] J. Yamashita, H. Itoh, M. Hirashima, *Nature* **2000**, *408*, 92.

[31] Y. J. van der Zijpp, A. A. Poot, J. Feijen, *Arch. Physiol. Biochem.* **2003**, *111*, 415.

[32] O. E. Teebken, A. Haverich, *Eur. J. Vasc. Endovasc. Surg.* **2002**, *23*, 475.

[33] Y. Tamada, E. A. Kulik, Y. Ikada, *Biomaterials* **1995**, *16*, 259.

[34] S. L. Rose, J. E. Babensee, *J. Biomed. Mater. Res. A* **2005**, *72*, 269.

[35] S. Kaushal, G. E. Amiel, K. J. Guleserian, O. M. Shapira, T. Perry, F. W. Sutherland, E. Rabkin, A. M. Moran, F. J. Schoen, A. Atala, S. Soker, J. Bischoff, J. E. Mayer, Jr., *Nat. Med.* **2001**, *7*, 1035.

_____