



HAL
open science

Degradation and healing characteristics of small-diameter poly(epsilon-caprolactone) vascular grafts in the rat systemic arterial circulation.

Erman Pektok, Benjamin Nottelet, Jean-Christophe Tille, Robert Gurny, Afksendiyos Kalangos, Michael Moeller, Beat H. Walpoth

► **To cite this version:**

Erman Pektok, Benjamin Nottelet, Jean-Christophe Tille, Robert Gurny, Afksendiyos Kalangos, et al.. Degradation and healing characteristics of small-diameter poly(epsilon-caprolactone) vascular grafts in the rat systemic arterial circulation.. *Circulation*, 2008, 118 (24), pp.2563-70. 10.1161/CIRCULATIONAHA.108.795732 . hal-00416599

HAL Id: hal-00416599

<https://hal.science/hal-00416599>

Submitted on 12 Jan 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Degradation and Healing Characteristics of Small-Diameter Poly(ϵ -Caprolactone) Vascular Grafts in the Rat Systemic Arterial Circulation

Erman Pektok, MD*; Benjamin Nottelet, PhD*; Jean-Christophe Tille, MD, PhD; Robert Gurny, PhD; Afksendiyos Kalangos, MD, PhD, FECTS; Michael Moeller, PhD; Beat H. Walpoth, MD

Background—Long-term patency of conventional synthetic grafts is unsatisfactory below a 6-mm internal diameter. Poly(ϵ -caprolactone) (PCL) is a promising biodegradable polymer with a longer degradation time. We aimed to evaluate in vivo healing and degradation characteristics of small-diameter vascular grafts made of PCL nanofibers compared with expanded polytetrafluoroethylene (ePTFE) grafts.

Methods and Results—We prepared 2-mm-internal diameter grafts by electrospinning using PCL ($M_n=80\,000$ g/mol). Either PCL ($n=15$) or ePTFE ($n=15$) grafts were implanted into 30 rats. Rats were followed up for 24 weeks. At the conclusion of the follow-up period, patency and structural integrity were evaluated by digital subtraction angiography. The abdominal aorta, including the graft, was harvested and investigated under light microscopy. Endothelial coverage, neointima formation, and transmural cellular ingrowth were measured by computed histomorphometry. All animals survived until the end of follow-up, and all grafts were patent in both groups. Digital subtraction angiography revealed no stenosis in the PCL group but stenotic lesions in 1 graft at 18 weeks (40%) and in another graft at 24 weeks (50%) in the ePTFE group. None of the grafts showed aneurysmal dilatation. Endothelial coverage was significantly better in the PCL group. Neointimal formation was comparable between the 2 groups. Macrophage and fibroblast ingrowth with extracellular matrix formation and neoangiogenesis were better in the PCL group. After 12 weeks, foci of chondroid metaplasia located in the neointima of PCL grafts were observed in all samples.

Conclusions—Small-diameter PCL grafts represent a promising alternative for the future because of their better healing characteristics compared with ePTFE grafts. Faster endothelialization and extracellular matrix formation, accompanied by degradation of graft fibers, seem to be the major advantages. Further evaluation of degradation and graft healing characteristics may potentially lead to the clinical use of such grafts for revascularization procedures.

Key Words: bypass ■ coronary disease ■ endothelium ■ grafting ■ revascularization ■ tissue engineering

Small-diameter prostheses are needed for cardiac and peripheral revascularization procedures. Autologous arterial and venous grafts are by far the best options as small-diameter bypass grafts¹; however, they cannot be used in all patients because of several limitations (ie, vasospasm, limited length, poor quality, and prior use). Homografts degenerate fast in the arterial circulation and have been used very selectively.² Conventional vascular prostheses such as expanded polytetrafluoroethylene (ePTFE) and polyethylene-terephthalate (Dacron) grafts have been used clinically for decades, but they are prone to thrombosis and intimal hyperplasia.³ Their long-term results are unsatisfactory below 6 mm of internal diameter.^{3,4}

Clinical Perspective p 2570

To date, several biodegradable polymers such as polyglycolic acid,^{5,6} polylactic acid, polydioxanone,⁷⁻⁹ and polyurethane¹⁰ have been investigated to serve as vascular scaffolds. The main principle is to create a temporary scaffold through which in vivo tissue ingrowth can replace the prosthesis, leaving a complete biological vascular conduit in due time.¹¹ In addition, such scaffolds should be biocompatible, compliant, easily processable, economical, and shelf ready for use as a vascular prosthesis.⁴ So far, no biodegradable small-diameter vascular prosthesis made of such biodegradable polymers has reached clinical use in the arterial circulation.

From the Departments of Cardiovascular Surgery (E.P., A.K., B.H.W.) and Clinical Pathology (J.-C.T.), University of Hospital of Geneva, Faculty of Medicine, Geneva, and Department of Pharmaceutics and Biopharmaceutics, School of Pharmaceutical Sciences, University of Geneva and University of Lausanne (B.N., R.G., M.M.), Switzerland.

*Drs Pektok and Nottelet contributed equally to this study.

Correspondence to Erman Pektok, MD, Department of Cardiovascular Surgery, University Hospital of Geneva, 24 Rue Micheli-du-Crest, 1211, Geneva 14, Switzerland. E-mail Erman.Pektok@hcuge.ch

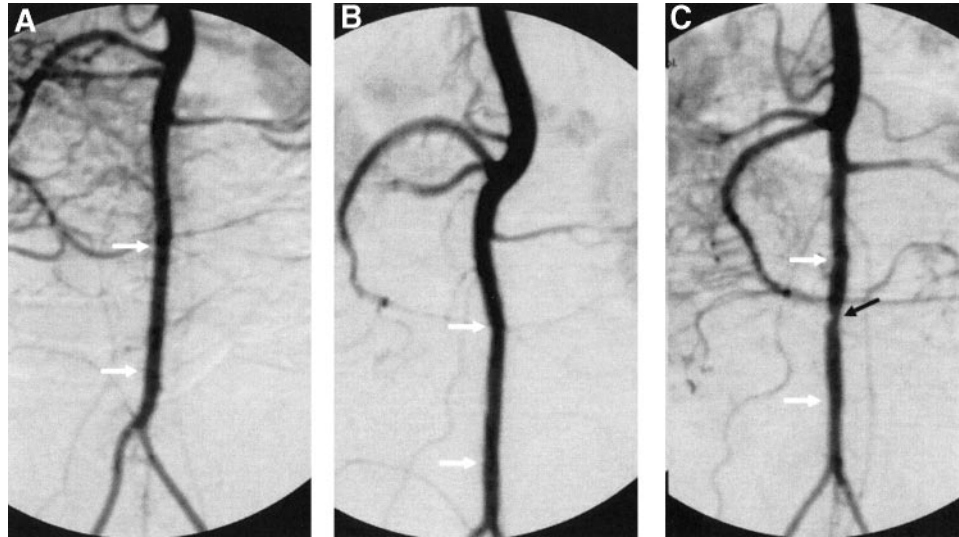


Figure 1. Monoplanar (anteroposterior) images of digital subtraction angiography 24 weeks after implantation. A, PCL graft. B, ePTFE graft without stenosis. C, ePTFE graft with a stenotic lesion (50%) in the midportion. White arrows indicate proximal and distal anastomoses; black arrow indicates the midgraft stenosis.

Poly(ϵ -caprolactone) (PCL) is a well-known biodegradable polymer with longer degradation time compared with other polymers.^{12–14} To date, it has been used clinically as a long-term contraceptive device (Capronor, Research Triangle Inst, Durham, NC) and for meniscus¹⁵ and bone¹⁶ reconstruction in animals. Recently, some groups have published their in vitro results with 3-dimensional vascular scaffolds made of PCL. Vaz¹⁷ fabricated 2-mm-internal diameter grafts with 2 layers of nanofibers: PCL on the luminal side and polylactic acid on the adventitial side. Pham et al¹⁸ used the same approach and fabricated bilayer grafts with PCL microfibers on the luminal side and PCL nanofibers on the adventitial side. Both groups used electrospinning to produce their fiber-based scaffolds. This technique allows the preparation of an extracellular matrix-mimicking structure, which should be beneficial for in situ tissue regeneration. However, they focused mainly on characterization and optimization of the

production technique, and no in vivo results have been published.

The results of our in vitro optimization process, including the initial in vivo feasibility, have recently been published.¹⁹ In the present study, our aims were to prepare biodegradable PCL-nanofiber grafts with optimal mechanical properties and to evaluate their in vivo healing and degradation characteristics in the rat arterial circulation at up to 6 months compared with conventional ePTFE vascular grafts.

Methods

Preparation of Vascular Grafts

Grafts (2-mm internal diameter, 13-mm length) made of PCL ($M_n=80\,000$ g/mol, $M_w=120\,000$ g/mol) were prepared by electrospinning as previously described in detail.¹⁹ Briefly, these grafts were obtained by use of a 15% (wt/vol) PCL solution in $\text{CHCl}_3/\text{EtOH}$ (7:3 vol:vol) and 6 minutes of spinning time, 12-mL/h flow

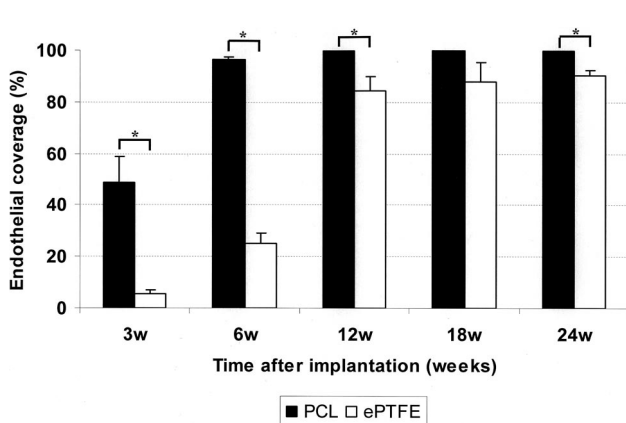


Figure 2. Endothelial coverage of PCL and ePTFE grafts calculated by computed morphometry. Data are expressed as mean \pm SEM. Endothelialization was significantly higher in the PCL group during the follow-up. $P=0.013$ at 3 weeks; $P=0.001$ at 6 weeks; $P=0.046$ at 12 weeks; $P=0.178$ at 18 weeks; and $P=0.007$ at 24 weeks. $*P<0.05$.

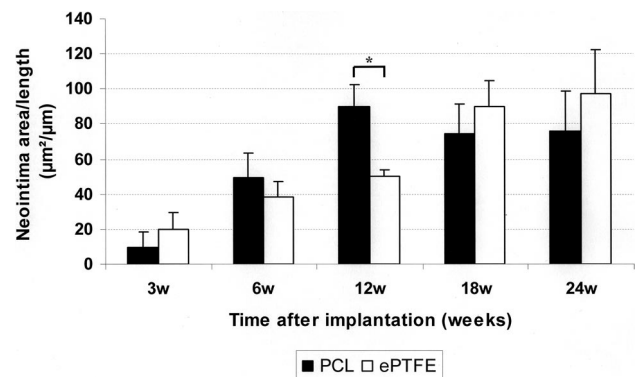


Figure 3. Neointima formation of PCL and ePTFE grafts calculated by computed morphometry. Data are expressed as mean \pm SEM. No statistically significant difference was found between the 2 groups with regard to neointima formation during the follow-up except at 12 weeks. Note the linear increase in neointima in the ePTFE group. $P=0.491$ at 3 weeks; $P=0.552$ at 6 weeks; $P=0.040$ at 12 weeks; $P=0.530$ at 18 weeks; and $P=0.563$ at 24 weeks. $*P<0.05$.

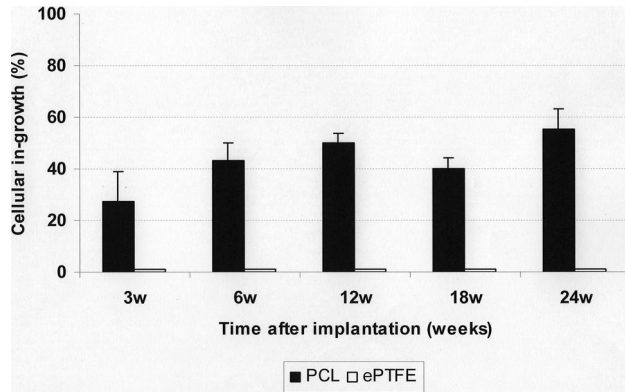


Figure 4. Transmural cellular ingrowth of PCL grafts calculated by computed morphometry. Data are expressed as mean \pm SEM. Note that cellular infiltration was not calculated in the ePTFE group because of a lack of cellular infiltration.

rate, 20-cm tip collector distance, and 20-kV voltage. All grafts were free of beads¹⁹ and had a mean fiber diameter of 1.90 μm , a tensile stress of 4.8 MPa, and a tensile strain of 600% after sterilization (gamma radiation, 25 kGy). The absence of residual solvent was confirmed in 3 sterile ready-to-implant grafts by the head-space-coupled GC detection technique. Commercially available ePTFE grafts (Atrium, 30- μm thru-pore, 2-mm internal diameter, 13 mm long) sterilized by ethylene oxide served as controls.

In Vivo Implantations, Follow-Up, and Sacrifice

Thirty male Sprague-Dawley rats (354 to 450 g) were operated on under isoflurane 2% mask anesthesia after induction with isoflurane 5%. The abdomen was shaved; a midline laparotomy incision was performed; and the infrarenal abdominal aorta was isolated by blunt and sharp dissection. After proximal and distal clamping, just below the renal arteries and above the aortoiliac bifurcation, a 1-cm segment of the abdominal aorta was resected in a bevelled way. Either PCL (n=15) or ePTFE (n=15) grafts were implanted randomly with 10-0 nylon interrupted sutures under sterile conditions using an operative microscope of $\times 25$ magnification. The abdominal cavity and skin were closed, and animals recovered from anesthesia and were kept in separate cages with normal food and water ad libitum. Rats were followed up for 3, 6, 12, 18, and 24 weeks (n=3 for each time point in each group). At the conclusion of the study period, all rats were anesthetized by intraperitoneal thiopental injection (40 mg/kg), and the left carotid artery was cannulated (24G intravenous cannula). Digital subtraction angiography (General Electric Cardiac Series, 9800, Salt Lake City, Utah) was performed in vivo, followed by explantation of the infrarenal segment of the abdominal aorta, including the implanted graft, and the animal was killed by pentobarbital overdose. The North American Symptomatic Carotid Endarterectomy Trial formula was used to calculate the degree of stenosis in the grafts. The experimental protocol was approved by the Animal Experiments Ethics Committee of the University of Geneva (protocol 06/52) and the Veterinary Office of State of Geneva (Switzerland; No. 1081/3232/11) and carried out in conformance with the *Guide for Care and Use of the Laboratory Animals* (National Research Council, Washington, DC: National Academy Press; 1996).

Histological and Quantitative Analyses

For histological investigations, explanted grafts with both anastomoses were fixed in 4% formaldehyde for 24 hours, cut into 2 longitudinal halves, and then embedded into paraffin. Histological sections (4 μm) were stained with hematoxylin and eosin (H&E), Miller and Masson for elastin fibers and collagen deposition, and anti-CD31 antibody (Santa Cruz Biotechnology Inc, Santa Cruz, Calif; PECAM-1 [M-20]) for endothelial cells and neovascularization. Slides were also analyzed quantitatively by computed histomor-

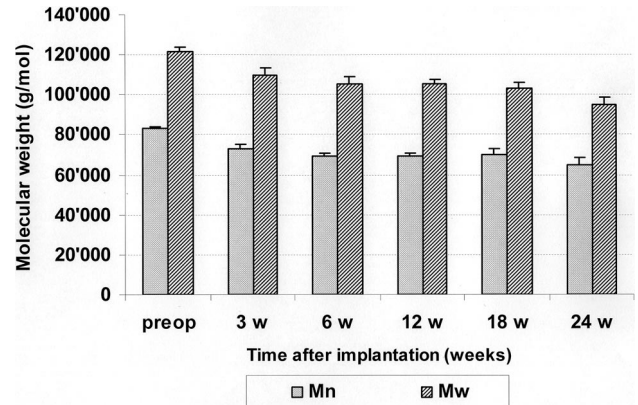


Figure 5. Molecular weight (Mn and Mw; g/mol) evolution of PCL grafts at up to 24 weeks of implantation measured by size exclusion chromatography and expressed as mean \pm SEM.

phometry with a Leitz Medilx (Leica, Nussloch, Germany) motorized microscope, a Sony 3CCD color video camera, and Leica Q-win software, standard version Y2.3 for image analysis. Endothelial coverage was defined as the length of endothelial cell layer on the luminal surface and was expressed as the percentage of total graft length. Neointima formation, defined as the area between the endothelial layer and luminal surface of the prosthesis, was calculated per unit length of neointima ($\mu\text{m}^2/\mu\text{m}$). Transmural cellular ingrowth was defined as the percentage of graft area penetrated densely by host cells from the adventitial tissue toward the luminal surface.

In Vivo Degradation Analysis of PCL Grafts

In vivo degradation of implanted PCL-based vascular grafts was assessed by molecular weight (M_n and M_w) analyses of explanted grafts. Molecular weight was measured by size exclusion chromatography with Waters equipment fitted with coupled Waters Styragel HR4 and HR3 columns as the stationary phase, tetrahydrofuran at 1-cm³/min flow rate as the mobile phase, and a Waters 410 refractometer as the detector. Typically, a small piece of fixed prosthesis was cut and residual surrounding tissues were removed with a surgical blade. PCL graft samples were then cut into very fine pieces dissolved in tetrahydrofuran and submitted to sonication for 30 minutes. The supernatant was filtered on a 0.20- μm Albet. Solid residues were further extracted in chloroform (CHCl_3), submitted to sonication for 30 minutes, and distilled off before tetrahydrofuran addition, filtration, and injection. Each sample was then injected in duplicate in both tetrahydrofuran and CHCl_3 . Outliers were eliminated using the Huber method in Minitab Statistical Software 15 (Minitab Inc), and \bar{V}_n and \bar{V}_w are given as the mean and SEM of 12 measurements with respect to polystyrene standards.

Statistical Analysis

Statistical tests were performed with SPSS (version 16.0; SPSS Inc, Chicago, Ill). Results are expressed as mean \pm SEM for continuous variables. The unpaired *t* test was used to compare nonparametric values. The level of significance was set at $P < 0.05$.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Animal Survival and Digital Subtraction Angiography Results

Sterile PCL grafts could be implanted easily into the rats using standard microsurgical techniques. All grafts were implanted by one surgeon (E.P.). Aortic clamp time was not different between the 2 groups (67.9 ± 1.4 minutes for the

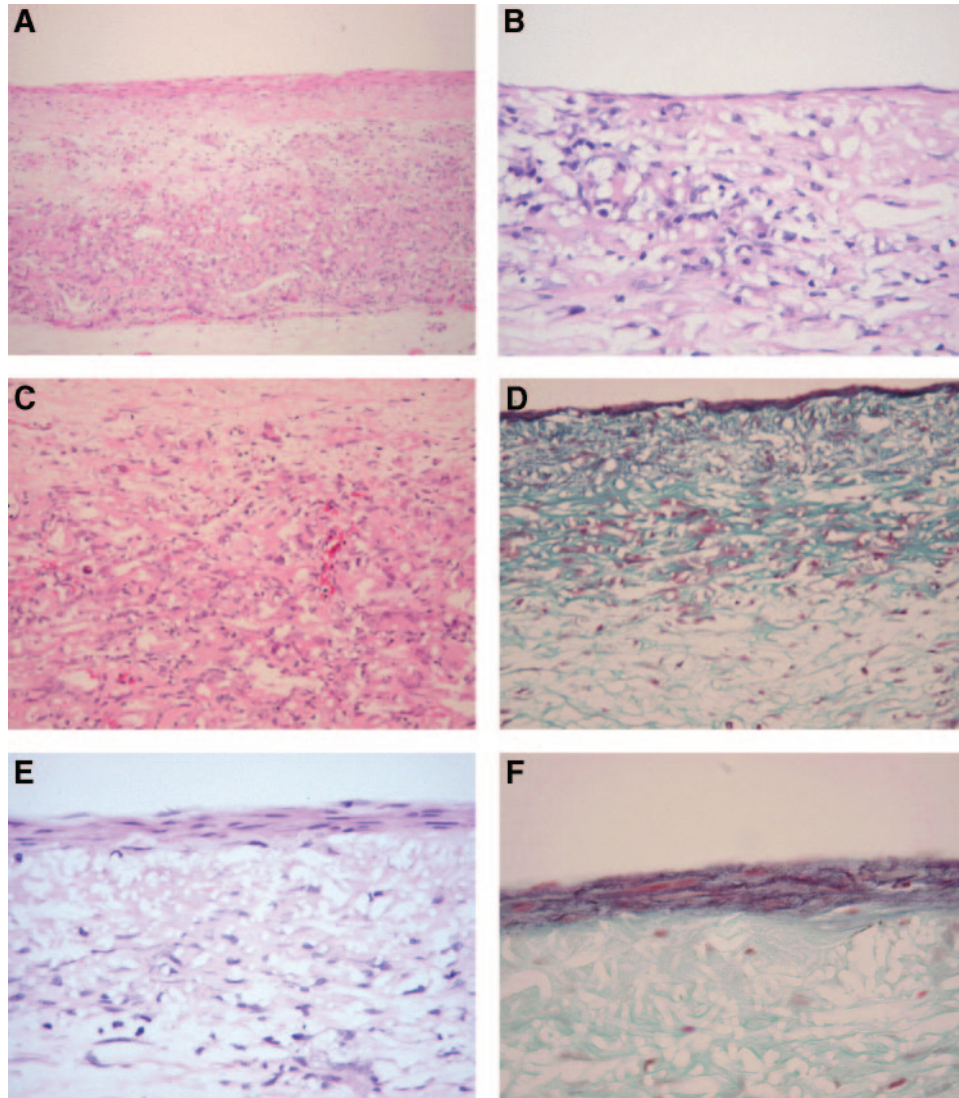


Figure 6. Histological analysis of PCL grafts in the midportion. A, Full thickness of the graft at 24 weeks showing homogeneous cellular infiltration (H&E staining; $\times 100$ magnification). B, Endothelialization of the luminal side of the graft at 12 weeks (H&E staining; $\times 400$ magnification). C, Macrophage reaction and neocapillary formation in the graft at 24 weeks (H&E staining; $\times 200$ magnification). D, Collagen deposition (green) in the graft without any elastin fibers (Miller and Masson staining; $\times 200$ magnification). E, Neointimal formation composed of few spindle-shaped cells at 6 weeks (H&E staining; $\times 400$ magnification). F, Elastin fibers in the neointima (Miller and Masson staining; $\times 400$ magnification).

PCL group, 72.1 ± 3.5 minutes for the ePTFE group; $P=0.27$). No significant bleeding was observed in either group. All animals survived until the end of follow-up, and all grafts were patent in both groups (Figure 1). Digital subtraction angiography revealed no significant stenosis in the PCL group (Figure 1A) but showed stenotic lesions in the midgraft region of 1 graft at 18 weeks (40%) and of another graft at 24 weeks (50%) in the ePTFE group (Figure 1C). No aneurysmal dilatation was found in either group.

Morphometry Analysis

Luminal endothelial coverage was longer in PCL grafts at all time points (Figure 2). Neointima formation increased gradually up to 12 weeks in the PCL group and reached a steady state after 3 months. Conversely, neointima formation was less pronounced but showed a persistent increase in the ePTFE group up to 24 weeks (Figure 3). Fifty percent of the graft

body was infiltrated by macrophages at 12 weeks in the PCL group, and this infiltration showed a tendency to increase at 24 weeks. However, ePTFE grafts were not infiltrated by macrophages, so a calculation could not be performed (Figure 4).

Molecular Weight Analysis for Degradation

Degradation analysis of the PCL prostheses revealed gradual loss of 20% for M_w and 22% for M_n at up to 24 weeks after implantation (Figure 5).

Histological Degradation and Healing of PCL Grafts

Histological analysis of PCL grafts revealed a rapid endothelialization of the luminal part of the graft, spreading from adjacent native aorta toward the graft body with a confluent monolayer of endothelial cells at 12 weeks (Figure 6A and

Table. Histological Findings During the Follow-Up

	ePTFE	PCL
Endothelial coverage	Incomplete at 24 wk	Confluent at 12 wk
Cellular infiltration	Weak cellular infiltration during the course of follow-up	Dense adventitial infiltration by macrophages ($\approx 25\%$ of graft at 3 wk, 50% after 12 wk)
Giant cell reaction around the graft	None	Adventitial monolayer of giant cells
Neovascularization	No neocapillary formation in the graft	Starting at 3 wk and increasing during the follow-up (along with cellular infiltration) throughout the graft
Neointima formation	Linear increase of neointima formation with significant ($\approx 40\%$ to $\approx 50\%$) stenotic lesions at 18 and 24 wk (1/3 at each time point)	A thin layer of neointima; no stenotic lesion at any time point
Chondroid metaplasia and calcification	No chondroid metaplasia but calcifications in the neointima near the proximal anastomoses at 24 wk	Chondroid metaplasia present at 6, 12, and 18 wk in the neointima; regression of chondroid metaplasia at 24 wk, which is replaced by calcification

6B). At the same time, we found a rapid and homogeneous colonization of the graft material by host cells consisting mostly of fibroblasts (the Table). No sign was seen of chronic inflammation. On the outer part of the graft, at the interface with the surrounding connective tissue, we found a giant cell reaction accompanied by macrophages. The degradation process triggered mainly by cellular infiltrates also existed on the outer part of the graft body, covering 50% to 60% of the graft body after 12 weeks (Figure 6A and 6C). Furthermore, some foci of degradation by macrophages were also found beneath the endothelium. Fibroblast density increased slightly with time and was accompanied by collagen deposition (Figure 6B and 6D). We also observed neocapillary formation in the graft body starting at 3 weeks and increasing with time (Figure 6C). Five to 7 layers of spindle-shaped cells and elastin fibers beneath the endothelium forming a neointima were observed after 3 weeks (Figure 6E). With time, this neointima formation covered 60% to 70% of the luminal surface of the graft without any increase in thickness containing elastin fibers (Figure 6F). However, as of 6 weeks of implantation, a basophilic matrix containing round chondrocytes with lacunae appeared in some of the neointimal regions and was defined as chondroid metaplasia (Figure 7A). At 12 weeks, this chondroid metaplasia started to disappear (Figure 7B), and residual calcifications were found at 24 weeks (Figure 7C). During the same period, spindle-shaped cells in the neointima were replaced by collagen accumulation.

Histological Healing Characteristics of ePTFE Grafts

Histological analysis of ePTFE grafts showed a delayed endothelialization, which was still incomplete at 24 weeks (Figure 8A and 8B). Very few fibroblasts had infiltrated the graft body at 3 weeks with no significant increase up to 24 weeks, no macrophage or giant cell reaction was found in the perigraft area, and a trace amount of neocapillary formation was observed around the ePTFE graft (the Table). However, we observed marked and inhomogeneous neointima formation with a steady increase in time in this group during the follow-up without any chondroid metaplasia formation (Figure 8C and 8D) but some calcific deposits near the anastomoses (Figure 8A). At 18 and 24 weeks, 2 ePTFE grafts had stenotic neointima formation (40% to 50%).

Miller and Masson staining revealed some collagen deposits in the ePTFE graft with sparse elastin fibers. These elastin fibers were observed in the neointima after 6 weeks and were then replaced by collagen at 24 weeks, similar to the PCL group (Figure 8D).

Discussion

During the last 2 decades, biodegradable materials have been a major interest in medical applications.^{12,20} The biostable materials have been replaced gradually by biodegradable materials in the field of tissue engineering. Among biodegradable polymers, polyglycolic acid, polylactic acid, polydioxanone, and PCL have been of special interest to many researchers because of their biocompatibility, wide range of mechanical properties, and biodegradation period, which make them suitable for use in the medical and pharmaceutical fields. Electrospun microfiber and nanofiber scaffolds made of such biodegradable polymers may serve as vascular grafts for tissue engineering applications because they have small pores, high porosity, high surface area, and small fiber sizes comparable to extracellular matrix of human tissues. Our previous experience with polydioxanone and polydioxanone-poly-lactic acid electrospun vascular grafts provided unsatisfactory results 12 weeks after implantation in the rat arterial circulation, showing significant aneurysmal dilatation caused by premature loss of strength,²¹ a major cause of graft failure with degradable polymers.²² PCL, a commercially available polyester with a slower degradation rate, is also well known for its good mechanical properties.¹² In our series, PCL grafts showed no aneurysmal dilatation at up to 6 months of follow-up, providing 100% patency rate. Premature structural deformities resulting from early degradation of the graft material seem not to be an issue for our electrospun PCL grafts because they keep their strength and prevent any aneurysm formation during the first 6 months despite the degradation of PCL chains with a molecular weight loss of $\approx 20\%$.

Endothelial coverage of the luminal surface, transmural cellular infiltration, and formation of neocapillaries in the graft body are the major graft healing characteristics. Additionally, degradation of graft material is an important issue for biodegradable vascular grafts.¹³ Early endothelialization of the luminal surface without excess neointima formation, enhanced cellular ingrowth, and angiogenesis into the graft

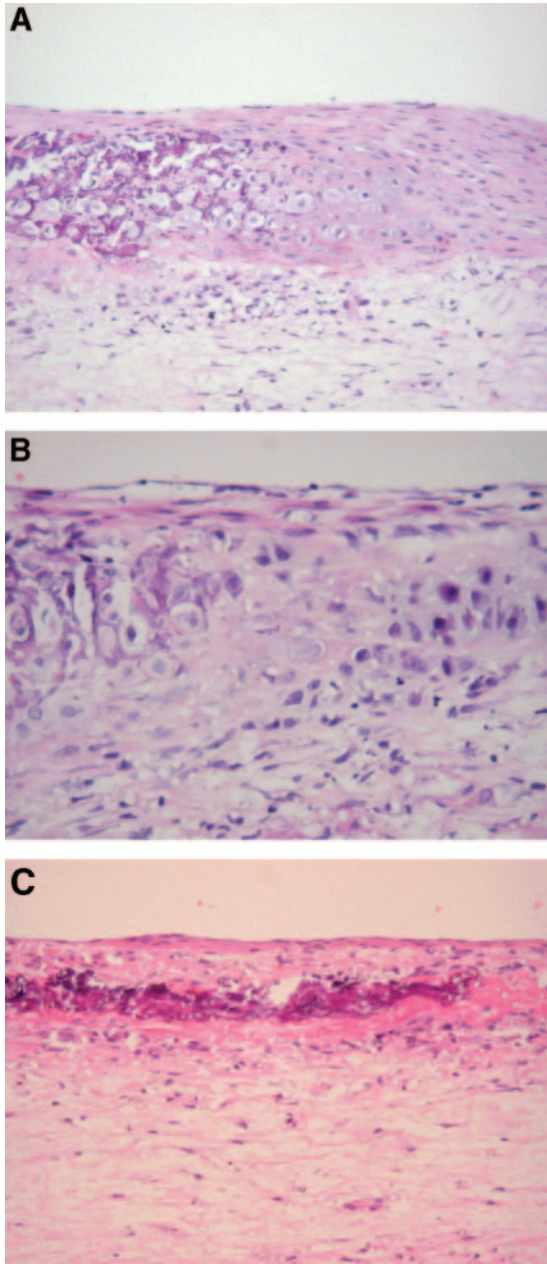


Figure 7. Chondroid metaplasia in the neointima formation of PCL grafts in the midportion at 6 weeks (A; H&E staining; $\times 200$ magnification) and at 12 weeks (B; H&E staining; $\times 400$ magnification). C, Chondroid metaplasia disappeared at 24 weeks, leaving some calcification behind (H&E staining; $\times 400$ magnification).

material without any inflammatory reaction potentially lead to increased biocompatibility of the implanted material and eventually better patency rates. Thus, we aimed to focus on healing of electrospun PCL vascular grafts under physiological circumstances. Moreover, ePTFE grafts are very inert, hindering graft-host interactions and graft healing and eventually leading to early graft failure. In our series, ePTFE grafts showed very poor healing compared with PCL grafts. We found that 97% of the luminal surface of PCL grafts was covered by endothelium 6 weeks after the implantation, and confluent endothelial coverage was achieved at 12 weeks.

Endothelialization of PCL grafts was significantly faster and better than that of ePTFE grafts in the follow-up. Neointima formation in the PCL group was homogeneous and leveled off 18 weeks after implantation. We speculate that this may be a consequence of the regulatory role of confluent endothelium.²³ Conversely, the ePTFE group showed incomplete endothelial coverage accompanied by a persistent increase in neointima formation with a nonhomogeneous pattern, creating stenotic lesions in 1 animal at 18 and in another at 24 weeks.

Degradation of PCL occurs mainly by nonenzymatic random hydrolytic cleavage of ester linkage.²⁴ Enzymatic degradation by lipases may also play a role to some extent,²⁵ but this enzyme has not been demonstrated in humans. At the latest stages of *in vivo* degradation, after the fragmentation of PCL chain into low-molecular-weight fragments, intracellular degradation can take place by phagocytosis.²⁶ This degradation process is faster *in vivo* than it is *in vitro*.^{13,20} Bölgen et al²⁰ implanted PCL patches ($M_n=51\,200$ g/mol, $M_w=84\,400$ g/mol) subcutaneously into rats, evaluated the degradation of material with time, and demonstrated a 30% reduction in molecular weight after 3 months. In our study, we found a 20% reduction in molecular weight at 6 months. Arterial pressure and blood flow might possibly affect the degradation and healing process. The former may cause deterioration of structural integrity by mechanical forces, leading to dilatation, turbulent flow, and/or thrombosis, and the latter may wash out acidic degradation products, enhancing the cellular infiltration and angiogenesis.¹³ Implanting PCL grafts into rat abdominal aorta resulted in deeper transmural cellular infiltration composed of macrophages, fibroblasts, and accompanying new capillaries, leveling off at 12 weeks after implantation. This cellular infiltration, accompanied by a monolayer of giant cells on the adventitial surface, which is part of chronic foreign body reaction, played a role in the degradation process soon after implantation and became clearly visible after 12 weeks (Figure 6). Obviously, PCL grafts were only partially degraded after 24 weeks. However, our aim was not to demonstrate the full degradability of PCL grafts but to evaluate their degradation and healing characteristics over time under dynamic *in vivo* conditions. In our study, the slower degradation rate of PCL grafts, which is preferable to maintain the functionality of the graft during the graft healing period, may be due to the use of higher- M_w and - M_n PCL.

Despite the favorable characteristics discussed above, it appears that chondroid metaplasia is the major drawback of these PCL grafts. Vascular smooth muscle cells have the potential of modification from a differentiated “contractile” phenotype to a dedifferentiated “synthetic” phenotype at sites of vessel injury or atherosclerosis,²⁷ where some hypoxia does exist.²⁸ Local hypoxia stimulates the overexpression of transforming growth factor- β_1 from the vascular smooth muscle cells and/or endothelium, which may induce chondroid metaplasia formation in the intima of the arterial wall.²⁹ We observed severe chondroid metaplasia in the neointima of our PCL grafts at 6, 12, and 18 weeks. These metaplastic areas were replaced by calcifications at 24 weeks. Although the mechanism is not yet clear, we believe that the local hypoxia accompanied by acidic degradation products trig-

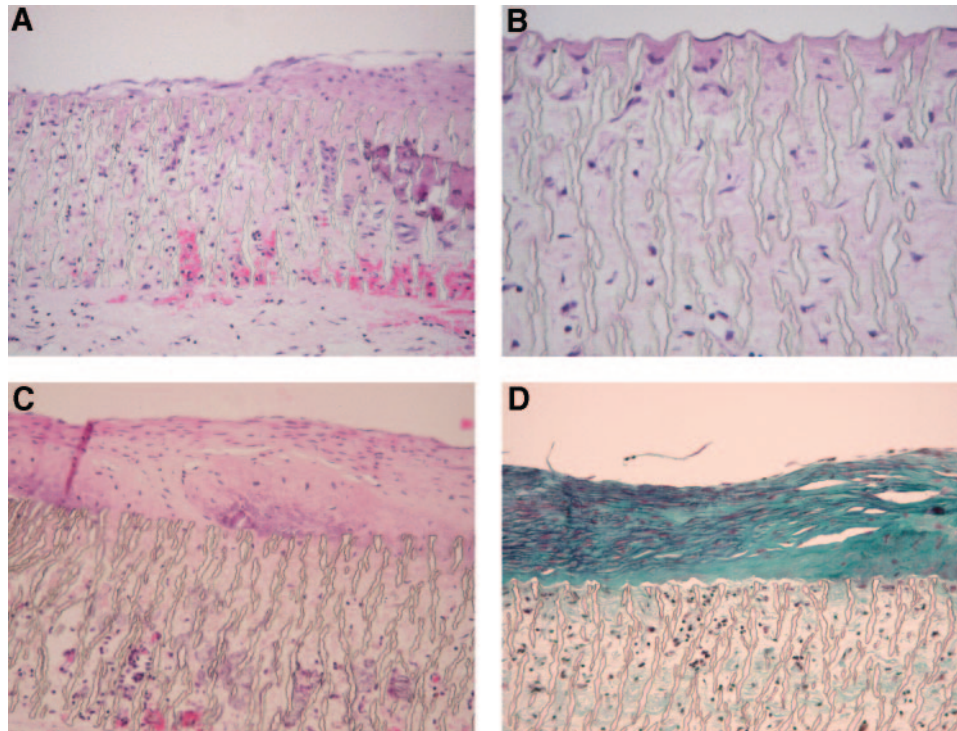


Figure 8. Histological analysis of ePTFE grafts in the midportion. A, Full thickness of the graft at 24 weeks showing sparse cellular infiltration and some calcification within the graft (H&E staining; $\times 200$ magnification). B, Endothelialization of the luminal side of the graft (H&E staining; $\times 400$ magnification). C, Neointima formation at 24 weeks with weak cellular ingrowth and collagen deposition (H&E staining; $\times 200$ magnification). D, Collagen deposition in the neointima and elastin fibers (Miller and Masson staining; $\times 200$ magnification).

gered some pathways as a local response and stimulated the expression of growth factors such as transforming growth factor- β_1 , leading to chondroid metaplastic degeneration.

The main limitation of our study was the small number of subjects at each time point and thus the limited statistical power. This was a consequence of the “3R principle” (reduce, refine, replace) in the animal experiments. However, the total number of rats was 15 per group, giving us the possibility of following up our end points (ie, endothelial coverage, neointima formation) longitudinally for up to 6 months and reaching statistical significance between nondegradable and degradable vascular grafts.

Conclusions

To the best of our knowledge, no data exist on the degradation and healing characteristics of PCL prostheses implanted into arterial circulation. In our experimental study with a 6-month follow-up, we found resistance to structural deterioration despite degradation and favorable graft healing characteristics, especially faster endothelialization. Thus, we hypothesize that PCL grafts may provide better patency rates than ePTFE grafts in revascularization procedures on small vessels. However, this has to be proven in large animal studies with longer follow-up before any clinical use. Further evaluation of degradation and graft healing characteristics, as well as preclusion of chondroid metaplastic degeneration, may potentially lead to clinical use of such biodegradable grafts for revascularization procedures in the future.

Acknowledgments

We would like to thank Professor Thomas Perneger (University Hospital of Geneva, Clinical Research Center, Geneva, Switzerland) for his contribution to statistical analysis and Melodie Kaeser, Unn Lutzen, Patrizia Gindre, and Catherine Biton from the University of Geneva for their precious support.

Disclosures

None.

References

- Tomizawa Y. Vascular prostheses for aortocoronary bypass grafting: a review. *Artif Organs*. 1995;19:39–45.
- Callow AD. Arterial homografts. *Eur J Vasc Endovasc Surg*. 1996;12:272–281.
- Pawlowski KJ, Rittgers SE, Schmidt SP, Bowlin GL. Endothelial cell seeding of polymeric vascular grafts. *Front Biosci*. 2004;9:1412–1421.
- Teebken OE, Haverich A. Tissue engineering of small diameter vascular grafts. *Eur J Vasc Endovasc Surg*. 2002;23:475–485.
- Greisler HP, Kim DU, Price JB, Voorhees AB. Arterial regenerative activity after prosthetic implantation. *Arch Surg*. 1985;120:315–323.
- Niklason LE, Gao J, Abbott WM, Hirschi KK, Houser S, Marini R, Langer R. Functional arteries grown in vitro. *Science*. 1999;284:489–493.
- Teebken OE, Pichlmaier AM, Haverich A. Cell seeded decellularised allogeneic matrix grafts and biodegradable polydioxanone prostheses compared with arterial autografts in a porcine model. *Eur J Vasc Endovasc Surg*. 2001;22:139–145.
- Cikirikcioglu M, Bowlin G, Cikirikcioglu YB, Djebaili K, Tatar T, Kalangos A, Walpoth BH. Biodegradable poly-dioxanone grafts for replacement of the abdominal aorta in the rat model. *Br J Surg*. 2005;92:915. Abstract.
- Smith MJ, McClure MJ, Sell SA, Barnes CP, Walpoth BH, Simpson DG, Bowlin GL. Suture-reinforced electrospun polydioxanone-elastin small-

- diameter tubes for use in vascular tissue engineering: a feasibility study. *Acta Biomater.* 2008;4:58–66.
10. van der Lei B, Wildevuur CR, Dijk F, Blaauw EH, Molenaar I, Nieuwenhuis P. Sequential studies of arterial wall regeneration in micro-porous, compliant, biodegradable small-caliber vascular grafts in rats. *J Thorac Cardiovasc Surg.* 1987;93:695–707.
 11. Xue L, Greisler HP. Biomaterials in the development and future of vascular grafts. *J Vasc Surg.* 2003;37:472–480.
 12. Nair LS, Laurencin CT. Biodegradable polymers as biomaterials. *Prog Polym Sci.* 2007;32:762–798.
 13. Sung H-J, Meredith C, Johnson C, Galis ZS. The effect of scaffold degradation rate on three-dimensional cell growth and angiogenesis. *Biomaterials.* 2004;25:5735–5742.
 14. Sun H, Mei L, Song C, Cui X, Wang P. The in vivo degradation, absorption and excretion of PCL-based implant. *Biomaterials.* 2006;27:1735–1740.
 15. Chiari C, Koller U, Dorotka R, Eder C, Plasenzotti R, Lang S, Ambrosio L, Tognana E, Kon E, Salter D, Nehrer S. A tissue engineering approach to meniscus regeneration in a sheep model. *Osteoarthritis Cartilage.* 2006;14:1056–1065.
 16. Mondrinos MJ, Dembzyński R, Lu L, Byrapogu VKC, Wootton DM, Lelkes PI, Zhou J. Porogen-based solid freeform fabrication of polycaprolactone-calcium phosphate scaffolds for tissue engineering. *Biomaterials.* 2006;27:4399–4408.
 17. Vaz CM. Design of scaffolds for blood vessel tissue engineering using a multi-layering electrospinning technique. *Acta Biomater.* 2005;1:575–582.
 18. Pham QP, Sharma U, Mikos AG. Electrospun poly(ϵ -caprolactone) microfiber and multilayer nanofiber/microfiber scaffolds: characterization of scaffolds and measurement of cellular infiltration. *Biomacromolecules.* 2006;7:2796–2805.
 19. Nottelet B, Pektok E, Mandracchia D, Tille JC, Walpoth B, Gurny R, Möller M. Factorial design optimization and in vivo feasibility of poly(ϵ -caprolactone)-micro- and nanofiber based small diameter vascular grafts. *J Biomed Mater Res A.* May 8, 2008. DOI: 10.1002/jbm.a.32023. Available at: <http://www3.interscience.wiley.com/cgi=bin/fulltext/119054365/HTMLSTART>. Accessed November 21, 2008.
 20. Bölgen N, Menceloglu YZ, Acatay K, Vargel I, Piskin E. In vitro and in vivo degradation of non-woven materials made of poly(ϵ -caprolactone) nanofibers prepared by electrospinning under different conditions. *J Biomater Sci Polym Ed.* 2005;16:1537–1555.
 21. Walpoth BH, Cikirikcioglu M, Mugnai D, Tille JC, Pektok E, Pfister R, Palombo D, Kalangos A, Bowlin G. Biodegradable small caliber polydioxanone-based vascular prostheses: potential as coronary bypass grafts. *Circulation.* 2007;116(suppl):II-444. Abstract.
 22. Walpoth BH, Bowlin G. The daunting quest for a small diameter vascular graft. *Expert Rev Med Devices.* 2005;2:647–651.
 23. Allaire E, Clowes AW. Endothelial cell injury in cardiovascular surgery: the intimal hyperplastic response. *Ann Thorac Surg.* 1997;63:582–591.
 24. Qian H, Bei J, Wang S. Synthesis, characterization and degradation of ABA block copolymer of L-lactide and ϵ -caprolactone. *Polym Degrad Stabil.* 2000;68:423–429.
 25. Li SM, Liu LJ, Garreau H, Vert M. Lipase-catalyzed biodegradation of poly(ϵ -caprolactone) blended with various polylactide-based polymers. *Biomacromolecules.* 2003;4:372–377.
 26. Woodward SC, Brewer PS, Moatamed F, Schindler A, Pitt CG. The intracellular degradation of poly(ϵ -caprolactone). *J Biomed Mater Res.* 1985;19:437–444.
 27. Iyemere VP, Prodfoot D, Weissberg PL, Shanahan CM. Vascular smooth muscle cell phenotypic plasticity and the regulation of vascular calcification. *J Intern Med.* 2006;260:192–210.
 28. Björnheden T, Levin M, Evaldsson M, Wiklund O. Evidence of hypoxic areas within the arterial wall in vivo. *Arterioscler Thromb Vasc Biol.* 1999;19:870–876.
 29. Schulick AH, Taylor AJ, Zuo W, Qiu CB, Dong G, Woodward RN, Agah R, Roberts AB, Virmani R, Dichek DA. Overexpression of transforming growth factor beta 1 in arterial endothelium causes hyperplasia, apoptosis, and cartilaginous metaplasia. *Proc Natl Acad Sci U S A.* 1998;95:6983–6988.

CLINICAL PERSPECTIVE

Finding better shelf-ready small-diameter grafts has been on the agenda of many researchers and clinicians for decades. Currently available expanded polytetrafluoroethylene and Dacron grafts have a very limited use under 6 mm of internal diameter in cardiac and vascular surgery, mainly because of early thrombosis and late intimal hyperplasia. Biodegradable polymers have recently been investigated to serve as vascular scaffolds. After optimization studies, we produced biodegradable small-diameter scaffolds made of poly(ϵ -caprolactone) nanofibers using electrospinning and implanted 15 poly(ϵ -caprolactone) and 15 expanded polytetrafluoroethylene grafts (2-mm diameter, 13-mm length) with a follow-up of up to 6 months. Our results showed that these poly(ϵ -caprolactone) grafts have significantly better healing characteristics such as faster endothelial coverage, better cellular infiltration accompanied by neoangiogenesis, and stable and homogeneous neointima formation compared with expanded polytetrafluoroethylene grafts. Rapid coverage of luminal surface by a confluent endothelium may prevent early graft thrombosis. In contrast to the expanded polytetrafluoroethylene grafts, which show increasing neointima formation and stenotic lesions over time, poly(ϵ -caprolactone) grafts showed a more homogeneous neointimal layer covered by confluent endothelium. This may prevent late graft occlusions resulting from intimal hyperplasia. However, this hypothesis has to be tested by studies that have long-term follow-up in the arterial circulation of animals. Using biodegradable scaffolds, this study adds data to the fields of vascular prostheses and tissue engineering. Our promising results are a step toward the shelf-ready coronary bypass grafts of the future.