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# Safety, Healing, and Efficacy of Vascular Prostheses Coated with Hydroxypropyl-β-cyclodextrin Polymer: Experimental *In Vitro* and Animal Studies

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**WHAT THIS PAPER ADDS**

- This study is a significant milestone in the development of drug-eluting polyester vascular prosthesis using a polymer of cyclodextrins. It addresses experimentally the safety and the efficacy of these prostheses before this innovative concept could be applied in clinical medicine. For that reason, this study is unique. The influence of this concept could be tremendous in the near future since one might be able to load specific bioactive molecules, especially antibiotics, onto a graft or stent graft according to therapeutic goals.

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A B S T R A C T

*Objectives:* Polyester vascular prostheses (PVPs) coated with a polymer of hydroxypropyl-β-cyclodextrin (HPβCD) have been designed to provide an *in situ* reservoir for the sustained delivery of one or more bioactive molecules. The goal of this study was to assess the efficacy, the safety and the healing properties of these prostheses.

*Methods:* Collagen-sealed PVPs were coated with the HPβCD-based-polymer (PVP-CD) using the pad–dry–cure textile finishing method and loaded with one or two antibiotics. Appropriate control and PVP-CD samples were tested in several *in vitro* and animal model conditions. The study end points included haemolysis, platelet aggregation, antibacterial efficacy, polymer biodegradation, acute toxicity and chronic tolerance.

*Results:* PVP-CD proved to be compatible with human blood, since it did not induce haemolysis nor influenced ADP-mediated platelet aggregation. Sustained antimicrobial efficacy was achieved up to 7 days against susceptible bacteria when PVP-CDs were loaded with the appropriate drugs. Analysis of harvested PVP-CD from the animal model revealed that the HPβCD-based coating was still present at 1 month but had completely disappeared 6 months after implantation. All grafts were patent, well encapsulated without healing abnormalities. Clinical data, blood-sample analysis and histological examination did not evidence any signs of acute or chronic, local or systemic toxicity in the animal models.

*Conclusion:* PVP-CD was proved safe and demonstrated excellent biocompatibility, healing and degradation properties. Effective antimicrobial activity was achieved with PVP-CD in conditions consistent with a sustained-release mechanism.

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The sealing of polyester (Dacron®) vascular prostheses (PVPs) is usually achieved with a collagen or a gelatine biodegradable matrix that degrades within 1–3 months of implantation.[1–3] During surgery, the sealing agent prevents blood permeation through the

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wall of the PVP.[1–3] There has been considerable investment in strategies designed to confer to those sealants specific therapeutic properties and to improve prosthetic graft outcomes. Disappointingly, this investment is yet to yield any great advance. Severe complications arise following prosthesis implantation despite constant refinements in surgical techniques and biomaterials. Infection is probably one of the most disastrous complications and is likely to occur in up to 6% of patients undergoing vascular surgery.[4–6] Given the small but potentially catastrophic risk of infection of implanted vascular grafts, their activation with antimicrobial agents targeted to specific pathogens has become an appealing concept to improve their resistance to infection. Similarly, development of such infection-resistant biomaterials would be of great value in instances of *in situ* reconstruction after removal of confirmed vascular infected grafts.[7,8]

Both native cyclodextrins (CDs) and their synthetic derivatives such as hydroxypropyl-β-cyclodextrin (HPβCD) are shaped as cage molecules with a hydrophobic internal cavity and a hydrophilic external surface.[9] Due to hydrophobic interactions with their internal part, they can encapsulate numerous active molecules, including antiseptics and antibiotics, and then release the preloaded molecules unmodified over a prolonged period of time. Formation of such complexes has been reported to result in delayed and sustained drug delivery.[10,11] The coating of PVP with a HPβCD-based-polymer (PVP-CD) is aimed to provide an *in situ* reservoir for the sustained delivery of one or more antimicrobials over several days.

Reported evidences of renal toxicity of native CDs when administered parenterally had however limited their use to non-parenteral administration routes.[12] The newer synthetic derivative HPβCD has demonstrated a significantly better safety profile and provides a novel opportunity for extending the use of CDs to various administration routes.[13] HPβCD has been extensively studied with regard to parenteral safety in a number of animal species and in humans, with no adverse effects documented to date on kidney function.[14] Despite this highly reported inocuity of HPβCD monomers, the safe use of PVP-CD remains to be ascertained. The goal of this study was to assess the efficacy, the safety and the healing properties of these prostheses.

## Material and Methods

### *Vascular prostheses*

Two types of manufactured PVP were provided by Perouse Medical (Ivry-Le-Temple, France): collagen-sealed weaved (Polythese®) and collagen-sealed knitted (Polymaille®) PVP. Both types were used either unmodified as commercially available or after HPβCD coating. The coating process was carried out using the pad–dry–cure textile finishing method, as previously described.[15] During the process, cross-linking of HPβCD moieties by citric acid (CTR) occurred though esterification reactions and resulted in a cross-linked CTR-HPβCD polymer that physically adhered to the PVP fibres. Both PVP-CD and uncoated-PVP were sterilised before use by gamma irradiation and stored in appropriate conditions (15–25 °C, safe from light and humidity).

### *Platelet aggregation assay*

Platelet-rich plasma (PRP) was prepared by centrifuging whole human blood from healthy volunteers at 1150 rpm for 15 min. Prior informed consent was obtained from every volunteer. The supernatant PRP was drawn off and the remaining blood centrifuged further at 4000 rpm for 15 min at room temperature to yield the platelet-poor plasma (PPP). PVP-CD (test article) and high-density polyethylene (control-article) were extracted with 0.9% sodium chloride (NaCl) for 24 h at 70 °C under agitated conditions (80 rpm) at a ratio of 3 $cm^2$ $ml^{-1}$. Test and control-article extracts were incubated with PRP at 37 °C for 5 min. Aggregation tests were conducted in the presence and absence of adenosine diphosphate (ADP, 5 mM). The aggregation profiles were monitored as a change in optical density for 5 min using an aggregometer calibrated with aggregation of PPP scored as 100 and PRP scored as zero.

### *Haemolysis test*

Haemolysis tests were conducted on leachables extracted from PVP-CD (test article) in accordance with the International Organisation for Standardisation (ISO) 10993-4 standards. To prepare immersion extracts, samples of the test article were incubated in Falcon tubes with Dulbecco's phosphate-buffered saline (DPBS) at 37 °C for 72 h under agitated conditions (80 rpm). The relation between the material surface and the volume of solution was 3 $cm^2$ $ml^{-1}$ as in the previous experiment. Falcon tubes without samples filled with DPBS only and incubated in the same conditions served as negative control. Blood samples were collected in ethylene diamine tetraacetic acid (EDTA) tubes from healthy donors and diluted in DPBS to obtain blood substrate containing 10 g $l^{-1}$ total haemoglobin. Prior informed consent was obtained from every donor. One millilitre portions of the resulting dilution were transferred to tubes containing the test-article extracts ($n = 3$) or the control vehicle (DBPS only; $n = 3$). Tubes were maintained in a stationary position during 3 h at 37 °C, with the exception of gentle inversions twice every 30 min. The suspensions were then centrifuged at 3250 rpm for 15 min and the plasma haemoglobin (free haemoglobin) was measured on the collected supernatants using a spectrophotometer at 540 nm. The Haemolytic Index (HI) calculated as follows determined haemolytic activity of the sample: HI (%) = free haemoglobin (g $l^{-1}$) × 8 × 100/total haemoglobin (g $l^{-1}$) in the diluted blood substrate. Data were expressed as the mean percentage ± SD of three separate experiments. HI will satisfy the standard of an implantable medical device when it is below 5%.[16]

### *Antibiotic loading*

PVP-CD specimens were immersed for 20 min at room temperature under agitated conditions (200 rpm) in an antibiotic aqueous solution just prior to the experiments. This resulted in loading the grafts with a significant amount of antibiotics as previously demonstrated.[5] Antibiotic solutions used during this study included rifampin (RFP; Rifadine® 600 mg, Sanofi-Aventis) at 60 g $l^{-1}$, vancomycin hydrochloride (VCY; Vancomycine 500 mg, Mylan) at 50 g $l^{-1}$, ciprofloxacine (CFX; 200 mg, Panpharma) at 2 g $l^{-1}$ or a combination of rifampin 60 g $l^{-1}$ plus ciprofloxacine 2 g $l^{-1}$ (RFP + CFX).

### *Antibacterial activity overtime*

Bacterial strains used to assess this activity were methicillin-resistant *Staphylococcus aureus* (MRSA) 07001 and *Escherichia coli* L70A4. Samples (6-mm diameter cut discs) of antibiotic-loaded PVP-CD were dipped individually in human blood plasma (Etablissements Français du Sang, Lille, France) for 7 days at 37 °C under agitated conditions (80 rpm). The plasma milieu was renewed every day during this time period. Thus, the antibiotics diffusing into the plasma medium would be sooner or later desorbed from the grafts as previously shown.[5] Bacteria were cultured from cryopreservative beads (−80 °C) onto inclined Müller-Hinton agar flasks at 37 °C for 24 h in aerobic conditions. A bacterial suspension was prepared in cysteinated one-quarter strength

Ringer's solution to a concentration of $10^5$ bacteria $ml^{-1}$. Müller-Hinton agar plates were then inoculated with this suspension (0.1 ml/plate). Fifteen minutes thereafter, samples in triplicates of each tested material were removed from human plasma, rinsed with DPBS and placed firmly in appropriate inoculated plates. This was carried out at $t_0$ and every 24-h period over the 7-day stay in human plasma. Each plate was subsequently incubated at 37 °C for 24 h aerobically. Zones of inhibition around the discs were measured as the mean diameter of the clear area surrounding a disc, in which bacteria were not able to proliferate. The results were plotted over time. Efficacy at any time point was noted if an inhibition radius of at least 10 mm was present.[17,18] This was conducted for MRSA and *E. coli* in two separate experiments.

### *Acute toxicity test*

Twenty naïve female Albino Swiss mice (*Mus musculus*) weighting 17–22 g, from Charles River Laboratories (L'Abresle, France), were used in this study. The animals were fed a regular rodent diet and allowed to acclimate for at least 5 days before beginning the experimental procedures. In accordance with ISO 10993-11, extracts of the prosthesis coated with the CTR-HPβCD polymer were prepared by immersion at 37 °C for 72 h in either 0.9%-NaCl solution or sesame oil under a ratio of 3 $cm^2$ $ml^{-1}$. The mice (five per group) were either injected intravenously with saline extracts (50 ml $kg^{-1}$) or intra-peritoneally with sesame oil extracts (50 ml $kg^{-1}$). The extraction vehicles without test article were similarly prepared and injected to serve as negative controls. Based on guidelines provided in the United States Pharmacopoeia, animals were weighted and observed for adverse reactions (Table 1) immediately and at 4, 24, 48 and 72 h after injection.[19]

### *Healing properties and chronic toxicity*

Twenty-four female mongrel dogs (*Canis familiaris*) weighting 23–30 kg, from Marshall BioResources (Lyon, France), were fed a regular diet and allowed to acclimate for at least 12 days before beginning the experiment. Two groups of dogs (Fig. 1) were bilaterally implanted in carotid arteries using 6-mm diameter PVP to construct an *in vivo* functional implantation model. Group-1 animals ($n = 12$) received randomly the PVP-CD on one side and the uncoated-PVP on the other. Group-2 animals ($n = 12$) received randomly the PVP-CD on one side and an antibiotic-loaded PVP-CD on the other (RFP, $n = 2$; CFX, $n = 2$; VCY, $n = 2$). This was carried out according to the European requirements (EEC directive 86/609) for the care and use of experimental animals. Moreover, the institutional ethic committee approved the study protocols.

Under general anaesthesia (induction, thiopental 10–15 mg $kg^{-1}$ intramuscularly; maintenance, $O_2$–$N_2O$–isoflourane 0.5–4%), exposure of right and left carotid arteries was performed. Systemic anticoagulation was achieved by a single injection of unfractionated sodium heparin (50 IU $kg^{-1}$). Each dog received cephalexin (30 mg $kg^{-1}$ $day^{-1}$) immediately and for 3 postprocedural days. The calibre of carotid artery external diameter was assessed and the artery clamped upstream and downstream before a 3–4 cm arterial segment was resected. The prosthesis was implanted into the gap with two end-to-end anastomoses constructed with a polypropylene suture. Closure was performed according to standard surgical practice and the incision was injected with butorphanol for postoperative analgesia. Animals were returned to individual cages receiving aspirin per os (75 mg $day^{-1}$) for 1 month and thoroughly examined daily. Blood samples were analysed (creatinine, alanine aminotransferase (AlAT), aspartate aminotransferase (ASAT) and bilirubin) to assess renal and hepatic functions at the implantation date, at 1 month and at 6 months. Animals were sacrificed by a lethal injection of barbiturate at 1 month (half of each group) or at 6 months.

**Table 1**
Criteria to assess acute systemic toxicity after intravenous or intraperitoneal injection in a mouse model.

| Grade | Response | Description |
|---|---|---|
| 0 | Normal | No adverse physical symptoms |
| 1 | Slight | Slight but noticeable symptoms: Hypokinesia, dyspnoea, abdominal irritation |
| 2 | Moderate | Symptoms definitely evident: Hypokinesia, dyspnoea, ptosis, diarrhoea, abdominal irritation, weight drops to between 15 and 17 g |
| 3 | Marked | Severe symptoms: Prostration, cyanosis, tremors, convulsion, abdominal irritation, diarrhoea, ptosis, dyspnoea, extreme weight loss (weight < 15 g) |
| 4 | Death | Mouse dies after injection |

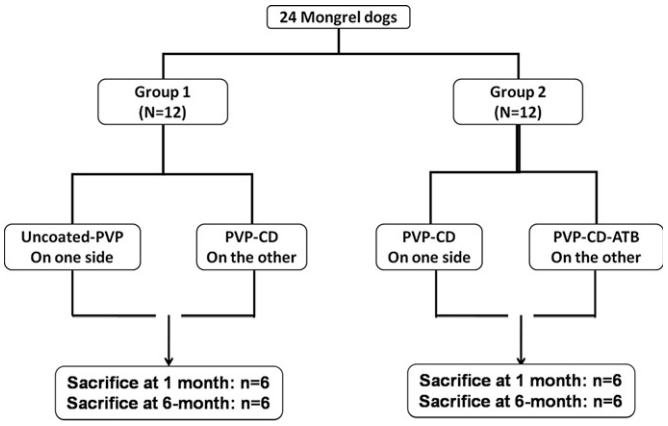


**Figure 1.** Flow-chart outlining the canine study design (PVP = polyester vascular prosthesis; PVP-CD = polyester vascular prosthesis coated with cyclodextrin; PVP-CD-ATB = polyester vascular prosthesis coated with cyclodextrin and loaded with antibiotics).

### *Macroscopic examination*

Local tolerance, graft patency and incorporation were assessed following animal euthanasia and harvesting of PVP. Gross examination of the implantation site, the implanted prosthesis and of all major organs (heart, lungs, liver, kidney and brain) was carried out followed by sampling any representative abnormal tissue. A 10%-buffered formalin solution was used to fix those specimens. Parameters assessed during gross examination are displayed in Table 2.

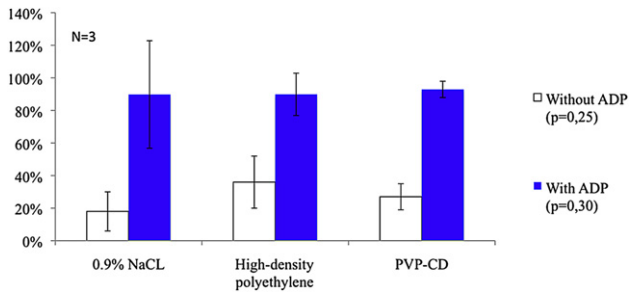


**Figure 2.** Platelet aggregation assay in the absence or presence of adenosine diphosphate (ADP) for PVP-CD (polyester vascular prosthesis coated with HPβCD), high-density polyethylene and 0.9% NaCl solution (data are expressed as mean ± SD). *P*-values are as determined for each condition by Kruskal–Wallis test.

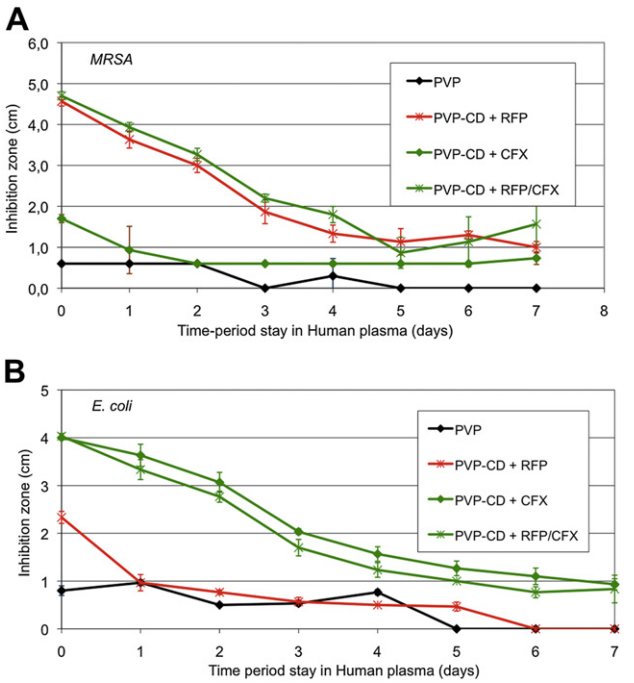


**Figure 3.** A: Antibacterial activity overtime ($N = 3$) of uncoated-polyester vascular prosthesis (PVP, control), of polyester vascular prosthesis coated with hydroxyl-propyl-β-cyclodextrin loaded with rifampin (PVP-CD + RFP), with ciprofloxacin (PVP-CD + CFX) or with rifampin and ciprofloxacin (PVP-CD + RFP/CFX) against *methicilin-resistant-Staphylococcus-aureus* (*MRSA*). *P*-values (PVP vs PVP-CD + RFP, $p < 0.0001$; PVP vs PVP + CFX, $p = 0.06$; PVP vs PVP-CD + RFP/CFX, $p < 0.0001$; PVP-CD + CFX vs PVP-CD + RFP, $p < 0.0001$; PVP-CD + CFX vs PVP-CD + RFP/CFX, $p < 0.0001$; PVP-CD + RFP vs PVP-CD + RFP/CFX, $p = 0.59$) are as determined by ANOVA followed by Fischer PLSD test. B: Antibacterial activity overtime ($N = 3$) of uncoated-polyester vascular prosthesis (PVP, control), of polyester vascular prosthesis coated with hydroxyl-propyl-β-cyclodextrin loaded with rifampin (PVP-CD + RFP), with ciprofloxacin (PVP-CD + CFX) or with rifampin and ciprofloxacin (PVP-CD + RFP/CFX) against *Escherichia coli* (*E. coli*). *P*-Values (PVP vs PVP-CD + RFP, $p = 0.34$; PVP vs PVP + CFX, $p < 0.0001$; PVP vs PVP-CD + RFP/CFX, $p < 0.0001$; PVP-CD + CFX vs PVP-CD + RFP, $p < 0.0001$; PVP-CD + CFX vs PVP-CD + RFP/CFX, $p = 0.37$; PVP-CD + RFP vs PVP-CD + RFP/CFX, $p < 0.0001$) are as determined by ANOVA followed by Fischer PLSD test.

### *Histological examination*

After fixation, biopsy samples from PVP and perigraft tissue were dehydrated in alcohol solutions of increasing concentration, cleared in xylene, embedded in paraffin and sectioned at 4–7 μm using a microtome. Two sections per specimen were stained with Masson's Trichome. Semiquantitative evaluation concerning the degree of inflammation was graded[20] from 0 to 4: absent '0', slight '1', moderate '2', marked '3' and severe '4' for each assessed parameter (Table 2).

### *CTR-HPβCD-polymer biodegradation*

Two-cm-length sections were recovered from each harvested PVP-CD. The amount of CTR-HPβCD-polymer remaining on these sections was assessed by the semiquantitative toluidine blue oxide (TBO) staining method[21] using a spectrophotometer at 634 nm. The TBO cationic dye would interact both with the COOH groups present on the CTR cross-links and with the HPβCD cavities such that amount of sorbed TBO and that of CTR-HPβCD-polymer were strictly proportional. Uncoated PVP and non-implanted PVP-CD specimens were used as controls.

### *Statistical analysis*

Statistical calculations were carried out using StatView® 6.0 software (SAS Institute, Cary, NC, USA). When comparing two groups, continuous variables were analysed with the Mann–Whitney *U*-test for unpaired data or with the Wilcoxon signed-rank test for paired data. For multiple comparisons or for repeated measures, the Kruskal–Wallis test or the analysis of variance (ANOVA) was used to compare the data as appropriate. Statistical significance was assumed at $p \leq 0.05$.

## Results

### *Platelet aggregation assay*

Fig. 2 displays the platelet aggregation rates for PVP-CD, control material and 0.9%-NaCl used as vehicle. There were no statistically significant differences between the tested samples either in

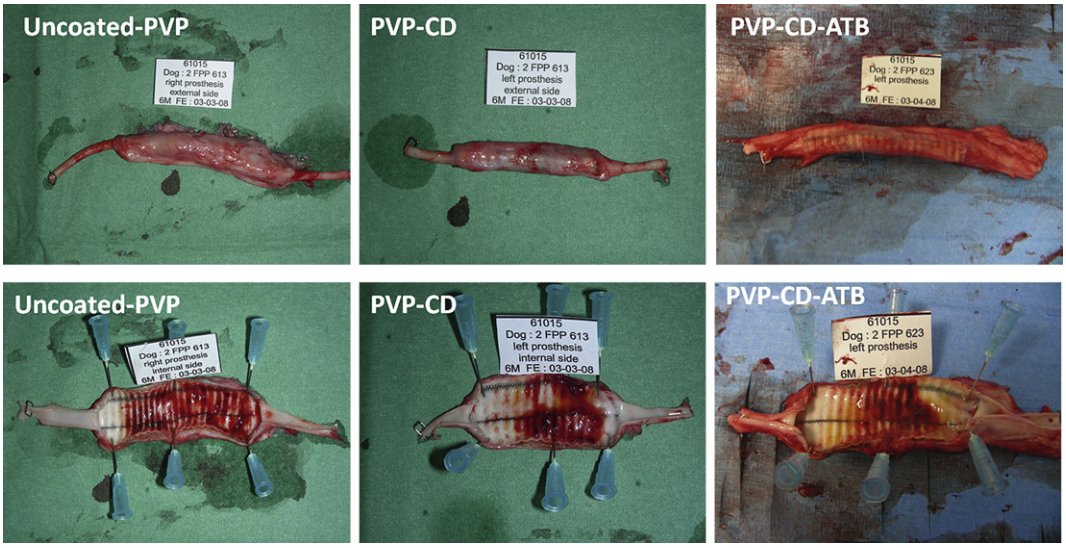


**Figure 4.** Macroscopic aspects of harvested polyester vascular prosthesis (PVP) coated or not with hydroxyl-propyl-β-cyclodextrin (PVP-CD; uncoated-PVP) loaded (PVP-CD-ATB) or not with antibiotics after 6-month implantation in a canine model of carotid bypass. Top: external surface showing further encapsulation and healing; bottom: luminal surface showing a slight grade of mural thrombus and the mismatch calibre between the graft and the native carotid artery.

**Table 2**
Assessed parameters and grade-scale for gross and histological examinations of harvested polyester vascular prosthesis or perigraft tissue samples in a functional canine-implantation model.

| Assessed parameters | | Semi-quantitative scale | | | | |
|---|---|---|---|---|---|---|
| Macroscopic evaluation | Microscopic evaluation | Grade 0 | Grade 1 | Grade 2 | Grade 3 | Grade 4 |
| Thrombogenicity | Thrombus | Absent | Slight | Moderate | Marked | Severe or total |
| Seroma | Fibrin | | | | | |
| | Polymorphonuclears | | | | | |
| Oedema | Macrophages | | | | | |
| | Lymphocytes | | | | | |
| Erythema | Plasma cells | | | | | |
| | Giant cells | | | | | |
| Necrosis | Necrosis | | | | | |
| Fibrous tissue development | Fibroblasts | | | | | |
| | Peri-implant fibrosis | | | | | |
| Tissular hyperplasia | Tissular integration | | | | | |
| | Calcification | | | | | |
| Graft dilatation | Implant degradation | | | | | |
| Neovascularisation | Neovascularisation | | | | | |
| Neointima | Endothelial-like cells | | | | | |
| | Myointimal hyperplasia | | | | | |

presence or in absence of ADP. Coating with HPβCD polymer neither induced platelet aggregation nor did it influence ADP-mediated platelet aggregation.

*Haemolysis test*

The mean HI for DPBS (negative control) and for the PVP-CD extracts was 0.63% ± 0.12% and 0.94% ± 0.4%, respectively ($p = 0.51$). PVP-CD likewise the negative control did not induce haemolysis when in contact with human blood (HI far less than 5%) and can be considered as highly haemocompatible.

*Antibacterial activity overtime*

In this experiment, the antimicrobial activity of different tested PVP supports over a 7-day period desorption in human plasma was assessed (Fig. 3(A) and (B)). The control material (uncoated-PVP) did not inhibit any bacterial proliferation. Declining but sustained antimicrobial efficacy (up to 7 days) was evidenced against MRSA with PVP-CD loaded with RFP (Fig. 3(A)), against *E. coli* with PVP-CD loaded with CFX (Fig. 3(B)) and against either bacterium with PVP-CD loaded with the RFP + CFX association. Neither synergistic nor antagonistic effects were depicted though (Fig. 3(A) and (B)), since one would expect with the antibiotic association greater or lesser activity, respectively. Of note, PVP-CD loaded with CFX only failed against MRSA while PVP-CD with RFP only failed against *E. coli*, both after the first-day desorption in human plasma.

*Acute toxicity test*

No mortalities or clinical evidence of acute systemic toxicity after PVP-CD extracts' injection were documented in mice that have all displayed a normal response throughout the study. Body weight gains of treated mice were similar to their respective controls (data not shown).

*Healing properties and chronic toxicity*

All PVP implantation procedures were successfully carried out. There were no perioperative deaths or signs of clinical abnormalities. Mean external carotid artery diameter was: 3.8 ± 0.3, 3.9 ± 0.2 and 4.0 ± 0.4 mm in PVP-CD, PVP-CD loaded with antibiotics and uncoated-PVP implantation sites ($p$ = NS), respectively. All grafts were patent at the end of the corresponding follow-up period and well encapsulated (Fig. 4). There were at 6 months some intraluminal thrombi allocated to the calibre mismatch between the 6-mm PVP and a 50% smaller carotid artery (Fig. 4). Findings from microscopic analysis of these thrombi were not consistent with infection and did not mandate any further microbiological examination.

Pooled data from macroscopic evaluation at termination are reported in Table 3 and showed that PVP-CD and uncoated-PVP were well tolerated locally and systemically at 1 month and at 6 months in all implanted animals. There were no grossly visible lesions in any major organ at animal sacrifice but one mongrel dog, implanted with uncoated PVP on one side and with PVP-CD loaded with RFP on the other side, was found dead at 4 months. Post-mortem histological analysis performed on this dog showed marked cerebral focal haemorrhagic necrosis, hepatic multifocal cholestasis, spleen red pulp hyperplasia and at both implantation sites a periprosthetic marked graded neutrophilic infiltration. All of these findings were consistent with intercurrent sepsis and probably related to the chronic multiresistant staphylococcal infection evidenced on bacteriological analyses. Of note, the involved *Staphylococcal aureus* strain was found only vulnerable to enrofloxacine, a CFX-like quinolone.

Data from blood-samples analysis throughout the study are displayed in Fig. 5(A)–(E). There was no impairment of renal or

**Table 3**
Local tolerance of different vascular prostheses[a] as assessed by gross examination[b] at 1 and 6 months post-implantation in a canine model.

| | Grade at 1-month | | | Grade at 6-month | | |
|---|---|---|---|---|---|---|
| | PVP | PVP-CD | PVP-CD +ATB | PVP | PVP-CD | PVP-CD +ATB |
| Erythema | 0 | 0 | 0 | 0 | 0 | 0 |
| Oedema | 0 | 0–1 | 1–2 | 0 | 0 | 0 |
| Seroma | 0–2 | 0–2 | 1–2 | 0 | 0–2 | 0 |
| Haemorrhage | 0–1 | 0–1 | 1–2 | 0–2 | 1–2 | 1–2 |
| Neovascularisation | 1–2 | 1–2 | 1–2 | 1–2 | 0 | 0–1 |
| Fibrosis development | 2–3 | 2–3 | 2–3 | 1–2 | 1–2 | 1 |
| Necrosis | 0 | 0 | 0 | 0 | 0 | 0 |
| Neointima formation | 0 | 0 | 0 | 1–2 | 0–1 | 1 |
| Tissular hyperplasia | 0 | 0 | 0 | 0 | 0 | 0 |
| Prosthetic dilatation | 0–2 | 0–2 | 1 | 0–1 | 0–1 | 0 |

[a] PVP: uncoated-polyester vascular prosthesis; PVP-CD: HPβCD coated-polyester vascular prosthesis; PVP-CD+ATB: HPβCD coated-polyester vascular prosthesis loaded with antibiotics.
[b] Gross-examination scale extends from 0 (absent), 1 (slight), 2 (moderate), 3 (marked) to 4 (severe or total) grade. Data are expressed as Mean ± SD.

hepatic function as reported in this figure. At 6 months, a non-significant rise in serum creatinine level was observed (compared with preoperative and 1-month values). The kidneys (Fig. 6) did not display any macroscopic abnormalities that would mandate further histological examination.

Prosthetic and implantation sites' histological examination did not reveal tissue alterations indicative of sub-acute (1 month) or chronic (6 month) toxicity for PVP-CD loaded or not with antibiotics as compared with uncoated-PVP (Tables 4 and 5). A slight to moderate grade of inflammatory infiltrate and fibrous tissue (Fig. 7(A)) was noted at 1 month around all three groups of PVP. At 6 months, myointimal hyperplasia (Fig. 7(B)) was graded slight for uncoated PVP and slight to moderate for PVP-CD loaded or not with antibiotics (Table 5).

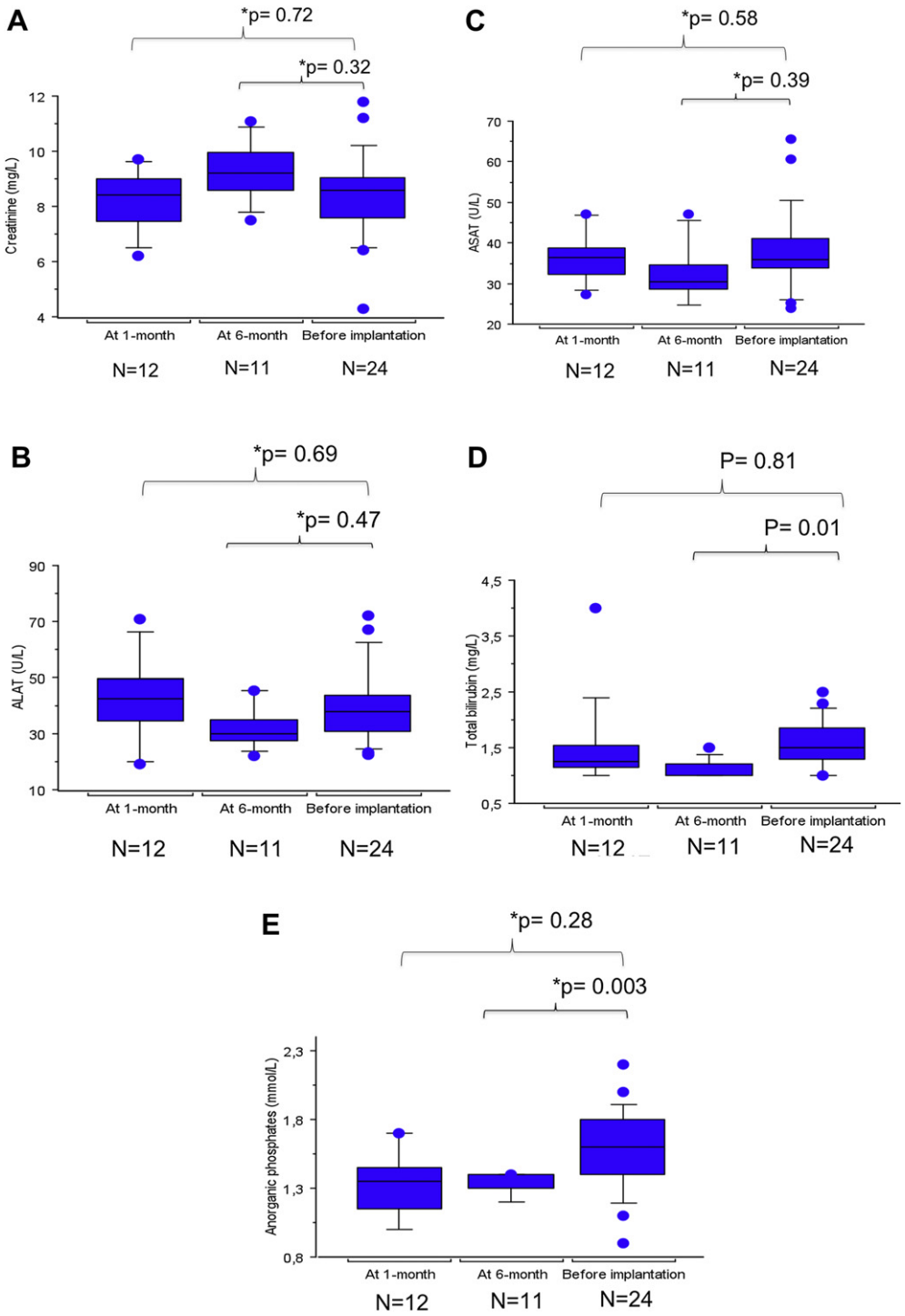


**Figure 5.** (A–E): Blood samples analysis results before implantation, at 1-month and at 6-months after implantation of polyester vascular prosthesis coated with hydroxyl-propyl-β-cyclodextrin in carotid position of mongrel-dogs. Data are expressed as the median with the inter-quartile range. *P-values as determined by Wilcoxon signed-rank test for values before implantation paired with those at 1-month or at 6-months. A: creatinine; B: ALAT; C: ASAT; D: total bilirubin and E: anorganic phosphates. All values are always within the expected normal clinical range despite statistical significance difference between time-periods sometimes.

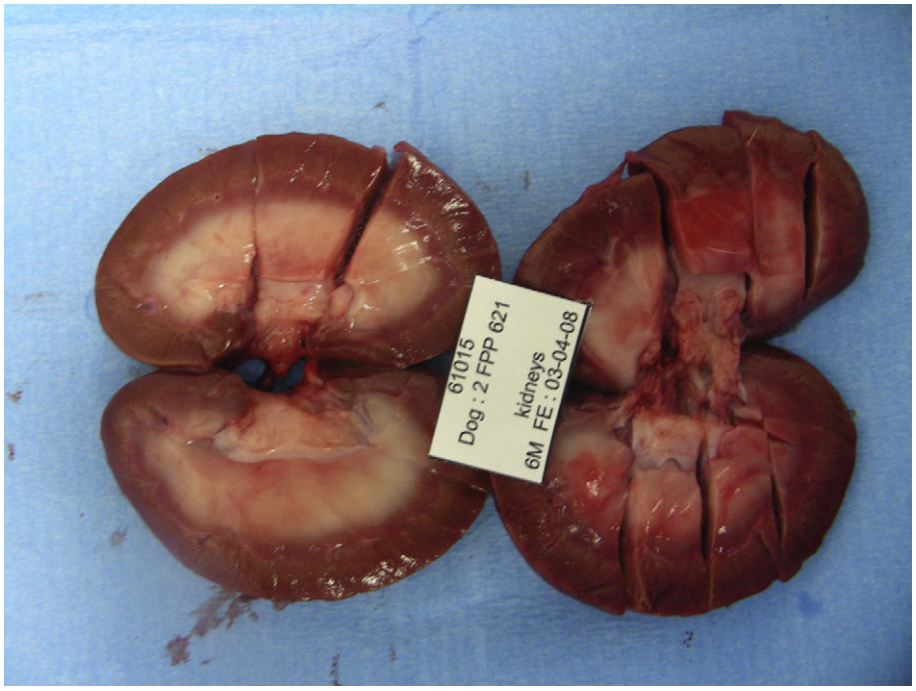


**Figure 6.** Macroscopic aspects of the kidneys (canine model) 6-months after PVP-CD implantation in carotid position.

*Biodegradation study*

The rate of sorbed TBO onto harvested PVP-CD was not changed at 1 month but at 6 months returned closely to the level observed in the uncoated PVP (Fig. 8). This suggested that CTR-HPβCD coating was still present after 1 month and had almost completely disappeared before the 6 month post-implantation.

## Discussion

Vascular implantable devices are ideal drug-delivery systems because they allow local delivery of active agents to the injured area, avoiding the need to deliver high doses systemically. Despite active and intensive research in cardiovascular biomaterials,[22] only coronary drug-eluting stents have reached routine clinical practice. Their current common usage highlights the potential benefits of pharmacologically functional implantable medical devices. The polyethylene terephthalate (PET) or e-polytetrafluoroethylene (e-PTFE) fabric of vascular grafts and stent grafts could offer a broader surface to use and a greater applicability than traditional bare stents. This experimental study represents a step further towards an effective drug-eluting PVP as a 7-day antimicrobial activity was achieved against susceptible bacteria.

**Table 4**
Semi-quantitative data of histological analysis[a] at 1-month post-implantation of vascular prosthesis[b] in a functional canine model of carotid bypass.

| | PVP | PVP-CD | PVP-CD+ATB |
|---|---|---|---|
| Fibrin | 1–2 | 1–2 | 1–2 |
| Patency | 4 | 4 | 4 |
| Thrombus | 0 | 0 | 0 |
| Necrosis | 0 | 0 | 0 |
| Polymorphonuclears | 0–1 | 0–1 | 0–2 |
| Lymphocytes | 0–1 | 0–1 | 0–1 |
| Plasma cells | 0 | 0 | 0 |
| Macrophages | 2 | 2 | 2 |
| Giant cells | 1 | 1 | 1 |
| Fibroblasts | 1 | 1 | 1–2 |
| Peri-Implant fibrosis | 1 | 1 | 1–2 |
| Neovascularisation | 1 | 1 | 1 |
| Calcification | 0 | 0 | 0 |
| Implant degradation | 0 | 0 | 0 |
| Endothelial-like cells | 1 | 1 | 1 |
| Neointimal thickness | 1 | 1 | 1 |
| Myointimal hyperplasia | 1–2 | 1–2 | 1 |
| Tissular integration | 1 | 1 | 1 |

[a] Histological examination scale extends from 0 (absent), 1 (slight), 2 (moderate), 3 (marked) to 4 (severe or total) grade. Data are expressed as Mean ± SD.
[b] PVP: uncoated-polyester vascular prosthesis; PVP-CD: HPβCD coated-polyester vascular prosthesis; PVP-CD+ATB: HPβCD coated-polyester vascular prosthesis loaded with antibiotics.

**Table 5**
Semi-quantitative data of histological analysis[a] at 6-month post-implantation of vascular prosthesis[b] in a functional canine model of carotid bypass.

| | PVP | PVP-CD | PVP-CD+ATB |
|---|---|---|---|
| Fibrin | 1–2 | 0–2 | 0–1 |
| Patency | 2–4 | 2–4 | 2–4 |
| Thrombus | 0–2 | 0–2 | 0–2 |
| Necrosis | 0 | 0 | 0 |
| Polymorphonuclears | 0–1 | 0–1 | 0–1 |
| Lymphocytes | 0–1 | 0 | 0–1 |
| Plasma cells | 0 | 0 | 0–1 |
| Macrophages | 1–2 | 1–2 | 1–2 |
| Giant cells | 0–1 | 1–2 | 1 |
| Fibroblasts | 2 | 2 | 1–2 |
| Peri-Implant fibrosis | 2 | 2 | 2 |
| Neovascularisation | 1 | 1 | 1 |
| Calcification | 0 | 0 | 0 |
| Implant degradation | 0 | 0 | 0 |
| Endothelial-like cells | 2 | 1–2 | 2–3 |
| Neointimal thickness | 2 | 1–2 | 1–2 |
| Myointimal hyperplasia | 1 | 1–2 | 1–2 |
| Tissular integration | 2–3 | 2–3 | 2–3 |

[a] Histological examination scale extends from 0 (absent), 1 (slight), 2 (moderate), 3 (marked) to 4 (severe or total) grade. Data are expressed as Mean ± SD.
[b] PVP: uncoated-polyester vascular prosthesis; PVP-CD: HPβCD coated-polyester vascular prosthesis; PVP-CD+ATB: HPβCD coated-polyester vascular prosthesis loaded with antibiotics.

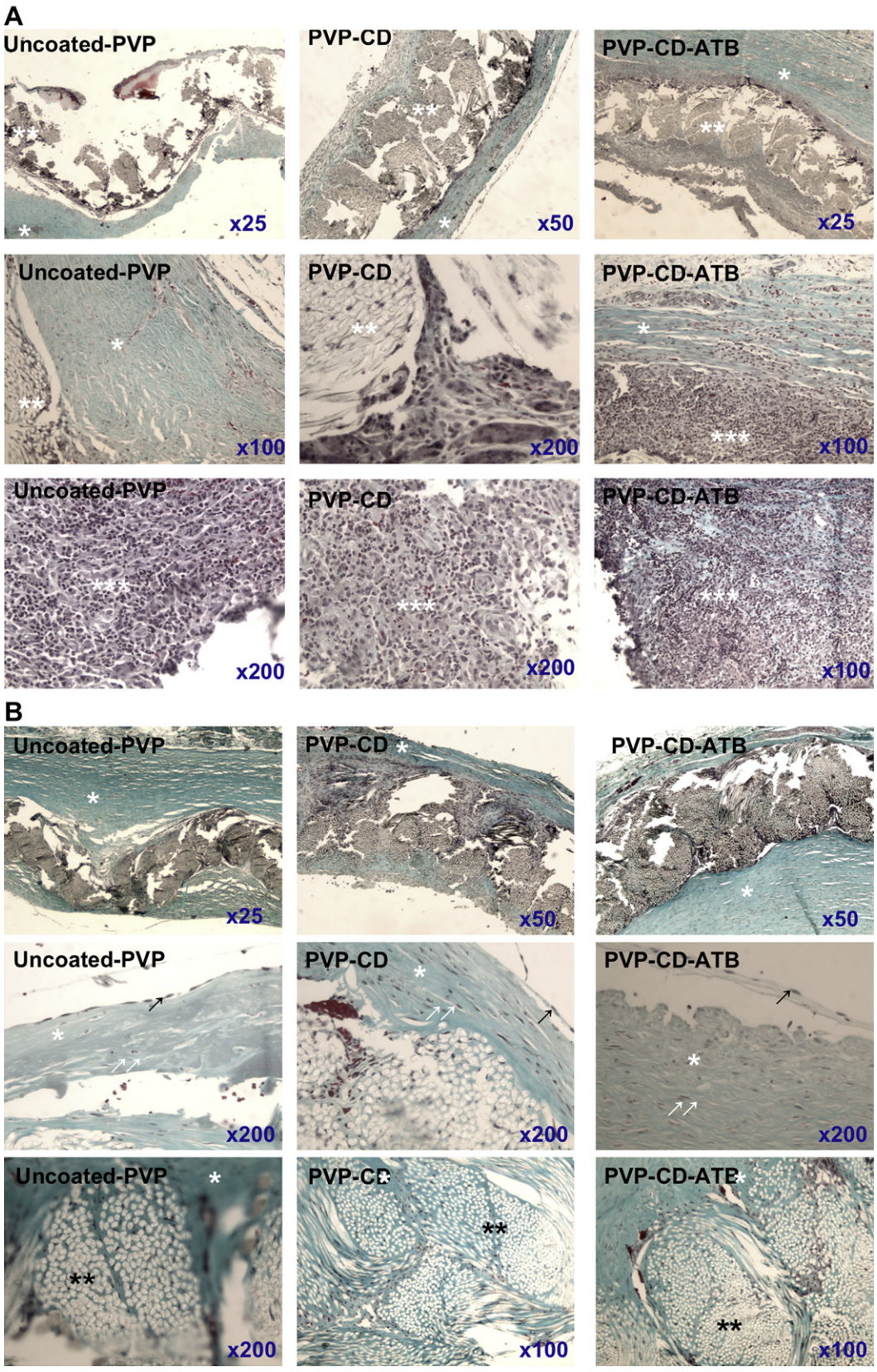


**Figure 7.** A: Histological analysis of implantation sites of polyester vascular prosthesis coated (PVP-CD) or not (uncoated-PVP) with hydroxyl-propyl-β-cyclodextrin loaded (PVP-CD-ATB) or not with antibiotics after 1-month implantation in a canine model of carotid bypass. Top: anastomotic site overview; middle: peri-implant fibrous tissue; and bottom: inflammatory infiltrate around the prosthesis. Masson's trichrome staining, light microscopy: *fibroblast proliferation; **prosthetic material; ***dense inflammatory infiltrate (Numerous neutrophils, very few monocytes). B: Histological analysis of implantation sites of polyester vascular prosthesis coated (PVP-CD) or not (uncoated-PVP) with hydroxyl-propyl-β-cyclodextrin loaded (PVP-CD-ATB) or not with antibiotics after 6-month implantation in a canine model of carotid bypass. Masson's Trichome staining, light microscopy showing: Top, implantation site overview; middle, the myointimal hyperplasia with sparse endothelial-like cells; and bottom, the tissular integration; (*fibroblast; **prosthetic material; ↗Endothelial-like cells; ↗↗ Smooth muscle cells). In polarized light microscopy some attempts of foreign material phagocytises were evidenced (slide not shown).

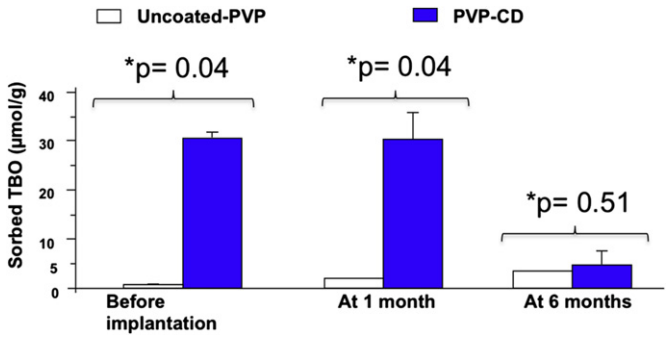


**Figure 8.** Biodegradation *in vivo* of the hydroxyl-propyl-β-cyclodextrin polymer after implantation of coated-polyester vascular prosthesis in the carotid artery of dogs. The amount of CTR-HPβCD polymer was measured as the toluidine blue oxide (TBO)-sorbed rate before implantation of the graft, at 1-month and at 6-month post-implantation. Uncoated polyester vascular prosthesis served as control. **P*-value as determined for each time period by Mann–Whitney test.

Our data demonstrated that implantation of PVP-CD is biocompatible and safe. There was no evidence of local or systemic toxicity. Within 6 months, PVP-CD was like uncoated PVP, well incorporated into surrounding tissues and without healing abnormalities. A mild inflammatory reaction did occur, but according to Murphy et al.,[23] neutrophils and monocytes normally migrate to sites of PVP implantation and are important in graft incorporation.

Only a very slight difference was noted at 6 months regarding myointimal hyperplasia between PVP-CD and uncoated-PVP samples, but within clinically acceptable range in both cases. Although mural thrombus was noted along the grafts, this was due to calibre mismatch and did not differ between PVP-CD and uncoated PVP. In addition, PVP-CD performed satisfactorily at platelet aggregation assay. Suspicion of infection-related thrombosis was similarly ruled out based upon histological data, although no further microbiological investigations were undertaken.

Safety[24] is a primary concern when considering new excipients intended for use in pharmaceutical formulations.[12] The literature provides many references demonstrating the use of CDs to enhance oral bioavailability of active compounds.[25] In this study, blood sample analysis and histological tests did not show any impairment of renal or hepatic function after PVP-CD implantation. This is consistent with the findings of the most recent investigations into CD polymers and CDs conjugated to other delivery vehicles, namely nanoparticles and liposomes.[26]

Native CDs are known to induce shape changes and membrane invagination on human erythrocytes, and eventually induce haemolysis at higher concentrations.[27] In this study, PVP-CD did not induce haemolysis in contact with human blood. The haemolytic activity of native CDs correlates with their inclusion ability towards membrane lipids rather than their intrinsic solubility or surface activity.[27] When the character of their lipophilic cavity is modified by chemical derivatisation (e.g., HPβCD), the effects on cell membranes may be tremendously modified.[12] Moreover, CDs lose their ability to interact with cell membranes when guest molecules occupy their cavities.[27] This supports further why PVP-CD loaded with a bioactive molecule should be unlikely to induce haemolysis in clinical applications.

Most grafts become contaminated by local flora during the initial placement procedure or within the weeks following implantation from adjacent or remote infection sites. The clinical onset may be instantaneous (<1 month), early (<4 months) or late (>4 months) depending whether the causative pathogen is highly virulent, virulent or relatively indolent. Both prolonged (>24 h) systemic prophylactic antibiotic treatment and local prophylactic rifampicin bonding to PVP did not prevent vascular graft infection after arterial reconstructions in a Cochrane systematic review.[28] Broad-spectrum systemic antibiotic prophylaxis may not be always adapted or may not reach the prosthetic implantation site at adequate concentration. Rifampicin is used locally without always taking into account the identity or antimicrobial susceptibilities of the involving organisms. Infections can also be polymicrobial. Most commonly implicated causative organisms produce biofilms with a barrier effect sustaining bacterial colonisation and protecting encased organisms from host defences and systemic antimicrobial therapy.[29] In this study, sustained antimicrobial efficacy was achieved *in vitro* up to 7 days against susceptible bacteria when PVP-CDs were loaded with the appropriate drugs. With this strategy, it might be possible to customise the local therapy to maintain for several days high and effective doses of one or more selected antimicrobial agents according to the ecology of the vascular graft infection. Further works are ongoing to assess this strategy with other antimicrobial agents and to possibly adapt it to antithrombotics, anticoagulants and mitotic spindle inhibitors. All these molecules could address common issues encountered after vascular graft and stent-graft implantation, namely patency, thombogenicity, myointimal hyperplasia, etc.

Potential limitations of this study may be related to the lack of an animal model to assess *in vivo* the sustained antimicrobial efficacy and to the lack of analyses using scanning electron microscopy. This would allow polymer integrity to be assessed after PVP-CD implantation, biofilm formation to be evaluated after bacterial contamination and incubation and interactions between bacteria and the HPβCD-based polymer to be determined.

## Conclusion

PVP-CD has been proved safe and demonstrated excellent biocompatibility, healing and degradation properties *in vitro* and in animal models. Effective antimicrobial activity was achieved *in vitro* with PVP-CD in conditions suggestive of a sustained-release mechanism. Further studies are needed to assess this drug-delivering strategy with other antimicrobial agents according to bacteria susceptibility or with other bioactive compounds depending on the therapeutic goals. Dedicated *in vivo* animal models are required to confirm the antimicrobial efficacy and to assess the clinical relevance of this drug-delivering strategy before specific trials of safety and efficacy could be undertaken in humans.

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## Conflict of Interest

None.

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