J Incl Phenom Macrocycl Chem (2007) 57:675–681
DOI 10.1007/s10847-006-9264-1

ORIGINAL ARTICLE

# In vitro study of a HP$\gamma$-cyclodextrin grafted PET vascular prosthesis for application as anti-infectious drug delivery system

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Received: 15 May 2006 / Accepted: 20 October 2006 / Published online: 20 January 2007


**Abstract** Hydroxypropyl-$\gamma$-cyclodextrin (HP$\gamma$-CD) was grafted onto woven polyester (PET) vascular prosthesis by using citric acid (CTR) as crosslinking agent. A polyCTR-HP$\gamma$ CD polymer was physically fixed onto the PET fibers. An optimal compromise between fixation temperature and fixation time was found and a grafting rate of 6.7% was obtained. The study of the inclusion of ciprofloxacin (CFX) and HP$\gamma$-CD was evidenced by using spectrophotometry. Sorption tests also showed that modified prosthesis could adsorb 5 times more CFX than the control. Biological tests revealed proliferation rates of human pulmonary micro-vascular endothelial cells (HPMEC) of 73 and 48% on virgin and modified prostheses respectively. We demonstrated that this was rather due to the increase of surface roughness of the fibers after their modification than to a toxic effect the polyCTR-HP$\gamma$CD polymer coating. Prostheses samples modified with HP$\gamma$CD and impregnated with CFX stayed up to 24 h in blood plasma. At various moments some aliquots were withdrawn from the medium and a positive antibacterial activity against *Staphylococcus epidermidis* was observed within the 24 h period for the grafted sample, whilst that of the virgin one had disappeared within 4 h. So, cyclodextrin coating of vascular prostheses may be suitable for the controlled release of CFX, and thus should help to the prevention of post surgery complications.

**Key words** Polyester vascular prosthesis · Cyclodextrins · Grafting · Biocompatibility · Endothelial cells · Microbiology · Ciprofloxacin · *Staphylococcus epidermidis*

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## Introduction

Vascular polyester prostheses are used to replace or bypass damaged arteries. After implantation, several complications may appear such as thrombi, aneurisms and infections. After implantation acts, an infection may occur in up to 6% of patients and can cause dramatic complications (antibiotic therapy for life, amputation, death) [1]. The most important germs described in these circumstances are *Staphylococcus aureus*, *Staphylococcus epidermidis, Pseudomonas* and other Gram Negative Bacteria (GNB) [2]. Many investigations were performed to reduce infections through the use of bactericide or antibiotic agents sorbed onto vascular prosthesis. The strategies

employed were *silver coating* (impregnation by a silver salt, Intergard Silver®, Intervascular) [3] or the coating with modified collagen impregnated with rifampicin (Gelweave® and Gelseal®, Vascutek) [4]. Goeau-Brissonniere et al. showed that the antibiotic (AB) treatment of the grafts was more efficient than *silver coating*. For instance, a study carried out on infected dogs showed that infections could not be reduced in 5/6 cases when silver coated prosthesis were used, whereas 100% success was obtained with prostheses loaded with rifampicin [5].

Nevertheless, in a general context, this method can be improved because the AB is too rapidly desorbed from the graft, and thus the protection period is too short and not significant.

In our approach, our strategy consisted to graft cyclodextrins (CDs) onto vascular grafts, in order, on one hand to enhance the sorption rate of the antibiotic, and on the other hand to achieve its controlled delivery. This system seamed to us to be relevant with regard to our goal which consisted to extend the antimicrobial activity period, and thus to protect patients against infections during the per and postoperative period.

The combination of ABs and CDs has been reported in the literature [6] with the aim to improve the solubility, stability and delivery of the AB to the targeted organ. At the end of 90's, Martel et al. showed the possibility to graft cyclodextrins by the mean of polycarboxilic acids on various synthetic textiles such as polypropylene, polyamide or polyester fabrics [7, 8]. Antibiotics and especially ciprofloxacin (CFX) are known to form inclusion complexes with CDs [9, 10].

So, The aim of this work was to demonstrate the possibility to functionalise vascular polyester prostheses with CDs, to establish their cytocompatibility, to investigate the complexation between CD and CFX and to verify the improved and prolonged antimicrobial effect of the graft in a synthetic biological medium.

## Materials and methods

Polyester (polyethyleneterephtalate, PET, Dacron® fibers) prostheses were manufactured by Laboratoires Perouse (Polythese®, Ivry-Le-Temple, France). Hydroxypropyl-$\gamma$-CDs (HP$\gamma$-CDs) was provided by Wacker Fine Chemicals GmbH (Cavamax®, Burghausen, Germany). Citric acid (CTR) was purchased from Aldrich chemicals (Milwaukee, Wi, USA). Ciprofloxacin (CFX) was a gift from Bayer Health Care (Ciflox®, Leverkussen, Germany).

The grafting process was based on the *pad-dry-cure* textile finishing method as previously reported [11, 12]: prostheses were impregnated by an aqueous solution containing HP$\gamma$-CD, catalyst and CTR and were roll-squeezed. Grafting occurred in a thermo-fixation oven (Roaches, UK) at variable temperature and time of fixation. The treated prostheses underwent a final washing step by soxhlet extractors with distilled water.

Virgin and modified PET prostheses were named respectively Polythese® and Polythese® HP$\gamma$-CD in the following text.

For biological and microbiological tests, disks of 15 mm in diameter were cut out from untreated and grafted PET prostheses. Negative and positive controls used in biological tests were respectively Thermanox® which is a treated polystyrene film with improved biological properties (Nunc International), and Nickel (Goodfellow, 99,999% pureness). Negative and positive controls used in microbiological tests were virgin PET disks impregnated or not with CFX.

The complexation between HP$\gamma$-CD and CFX was observed by spectrophotometry measurements (Nicolet® evolution 300, Thermo). The concentration of CFX solution was $2.41 \times 10^{-5}$ mol/l and HP$\gamma$-CD concentration varied from $2.41 \times 10^{-3}$ to $2.41 \times 10^{-2}$ mol/l. The mixtures were sonicated at 20°C for 20 min before use. CFX solutions presented three absorption bands situated at 275, 317 and 330 nm [13].

In vitro cell proliferation tests were performed with human pulmonary micro-vascular endothelial cells (HPMEC) [14], following the International and European standards (ISO 10993-5/EN 30993-5). HPMEC cells were cultivated in Endothelial Cell Growth Medium MV (Promocell), supplemented with 15% foetal calf serum (FCS) (Eurobio). All media contained streptomycin (0.1 g $l^{-1}$) and penicillin (100 IU $ml^{-1}$). All in vitro cell incubations were performed at 37°C in 5% $CO_2$ atmosphere and 100% relative humidity in a Binder $CO_2$ incubator (CB 150/APT.line) with high stability of all technical parameters.

The growth periods for cell proliferation tests were three and six days without renewal of the medium [15]. PET disks were placed at the bottom of 24-well plates (Nunc) and incubated with $10^4$ cells. After three and six days, cells were detached by addition of a trypsine-EDTA solution and were counted by using a cell counter Z1 (Coulter Electronics) instrument.

For SEM observations, cells were stained with 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer at pH 7. After two washes in the same buffer, they were post-stained with 1% $OsO_4$ in saturated $HgCl_2$, dehydrated in graded ethanol, critical point dried, and finally sputtered with gold/palladium. Surface of polyester fibres and cell morphology were observed with a SEM apparatus (JEOL J-SM-5300) using an

accelerating voltage of 30 kV and a current of 100 $\mu$A [16].

The total amount of sorbed CFX by virgin and CD finished prostheses was accomplished according to the following method.

Samples were preliminarily dipped into a 2 g/l CFX aqueous solution during 25 min. Then the total amount of sorbed CFX was carried out in two steps. Firstly prostheses samples were put in methanol (20 ml) at 37°C overnight. The CFX concentration in methanol was measured by spectrophotometry at 280 nm. Secondly, these samples were treated in a 0.1 M (20 ml) sodium hydroxide solution (4 h, 37°C), in order to hydrolyze the grafted polyCTR-HP$\gamma$CD polymer, and to release the rest of CFX that was still present on the prostheses into the solution. The concentration of CFX in the NaOH solution was determined by spectrophotometry at 271 nm. The addition of both spectrophotometric results allowed us to determine the total amount of CFX sorbed onto both samples [4].

Microbiological tests were performed according the standardized Kirby-Bauer method [17]. Aliquots cut off from prostheses with a 10 mm diameter disk shape were impregnated by a CFX solution (2 g/l) and dried. Samples were placed in batch systems in human plasma. (human blood provided by Etablissement Francais du Sang,–France) up to 24 h. Samples were withdraw from the plasma batch at regular intervals (2, 4, 8, 12 and 24 h) and placed in contact of a Agar gel (Muller Hinton) and inoculated with a 24 h culture of a *Staphylococcus epidermidis* strain recently isolated from a clinical sample. After one day of incubation at 37°C, a clear circular decontaminated zone appeared all around the prosthesis aliquot. The radius of this clear zone was measured and the ''inhibition radius'' was calculated as the difference between the radius of the circular clear zone minus that of the prosthesis aliquot (5 mm) . This values were then plotted against the time of presence in the plasma batch and were reported as the residual antimicrobial activity of the prosthesis.

## Results

### HP$\gamma$CD finishing of the prosthesis

The HP$\gamma$CD grafting assays were performed successively at 140, 150, 160 and 170°C at variable fixation times comprised between 0 and 60 min. The weight increase of the treated samples was measured (%wt) and was reported as the grafting rate. These experiments resulted to the diagram displayed in Fig. 1 which

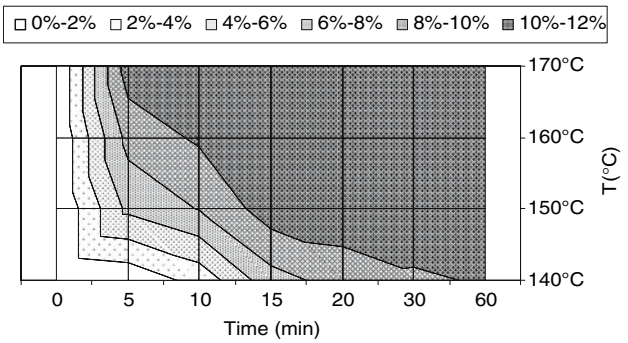


**Fig. 1** Influence of time and temperature on the grafting rate (%wt) on the PET supports

represents the grafting rate of the prostheses against time and temperature of fixation. As previously reported, [8], grafting was due to the occurrence of a polymerization reaction between HP$\gamma$CD and CTR, yielding poly-CTR-HP$\gamma$CD polymer that was physically anchored to the fibres network.

One can observe that maximal grafting rates could be reached in optimised conditions either by the choice of a high temperature and a low curing time, or on the contrary, by a low temperature and a long curing time. For example, at 140°C, a 30 min fixation time was necessary to obtain a maximum grafting rate of 10–12% wt, whereas 5 min were enough at 170°C to reach the same value. The former conditions (140°C, 40 min) were applied in order to prevent the graft from any thermal degradation and to maintain its optimal mechanical properties [18].

### Observation by microscopy

SEM examinations revealed a smooth surface of virgin Polythese® fibres (Fig. 2a) and a rough surface of grafted Polythese®HP$\gamma$-CD fibres due to the polyCTR-HP$\gamma$CD polymer coating (Fig. 2b).

### HP$\gamma$CD-CFX complexation study

The inclusion complex between cyclodextrins and CFX was observed by spectrophotometry measurements. Figure 3 shows an increasing absorption of the peaks of CFX at 275 nm after adding increasing amounts of HP$\gamma$-CD in the antibiotic solution. Moreover, the observation of isobestic points reveals the equilibrium between the free and the complexed forms of CFX in the solution. So, CFX and HP$\gamma$-CD can form host-guest species in solution. According to the Benesi-Hildebrand equation [13], the calculated association constant of the complex formed between HP$\gamma$-CD and CFX was 38 l/mol. From this statement, we extrapo-

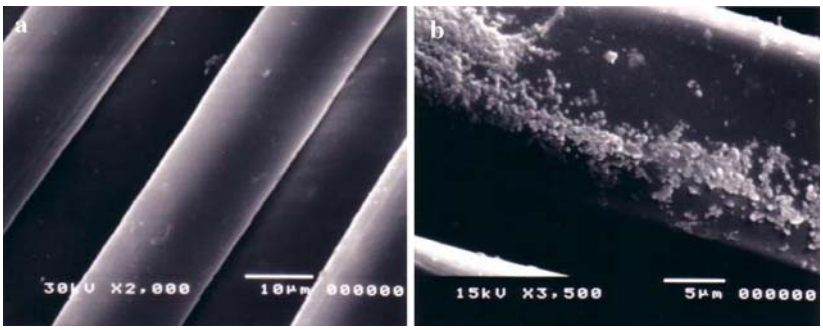


**Fig. 2** SEM observations of Polythese® (a) and Polythese®HP$\gamma$-CD (b)

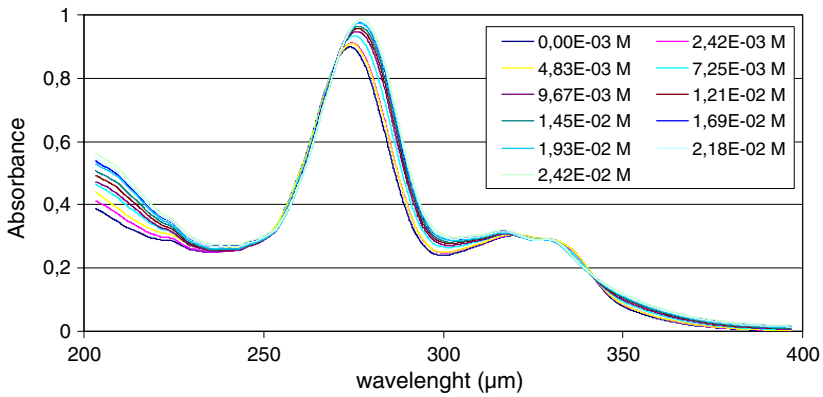


**Fig. 3** UV spectra of CFX in the presence of increasing concentration of HP$\gamma$-CD

lated that the inclusion phenomenon could occur between CFX and the CD cavities present on the polyCTR-HP$\gamma$CD grafted fibers. Though, we could not achieve the direct measurement of the inclusion complex constant in this heterogeneous system. Furthermore, other phenomena than inclusion complexation were also involved (physical adsorption) in the sorption of CFX onto the insoluble fabric supports.

## Study of the sorption of CFX

The affinity between CFX and Polythese®HP$\gamma$-CD was evidenced by the measurement and the comparison of the amount of sorbed CFX by virgin and grafted supports in batch system. One can observe in Fig. 4 that 3.9 and 22.0 mg/g were fixed respectively on both systems, this corresponded to a 5.5 fold improvement of the sorption capacity due to CD finishing. These data clearly demonstrate the role of polyCTR-HP$\gamma$CD in fixation of CFX onto the PET support.

Cell proliferation assays (Fig. 5) developed in direct contact with Nickel powder (toxic substrate towards living cells) induced low proliferation rates of HPMEC cells after 3 (16%) and 6 days (3%). Proliferation rates of endothelial cells on Polythese® were observed after 3 days (79%), and slightly decreased after 6 days (69%). Proliferation rates of 69 and 56% were observed on Polythese® HP$\gamma$-CD after respectively 3 and 6 days of incubation. The comparison between both series of results mentioned above indicated that the ranges of values of proliferation rates were sharply different and confirmed that polyCTR-HP$\gamma$CD coating polymer was not toxic against HPMEC.

SEM pictures (Fig. 6a, b) revealed that the endothelial cells present a similar morphology on Polythese® and Polythese® HP$\gamma$-CD after 3 days of incubation. Cells present on the textile support exhibit a globular form with few lamellipodia, pseudopodia and fillipodia. On the opposite, cells grown up onto a control present a spread form and more dense attachment sites (Fig. 6c). This shows that cell adhesion was

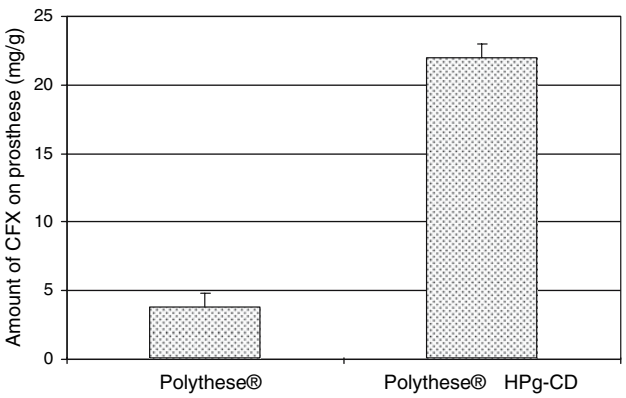


**Fig. 4** Sorbed amount of CFX (mg/g) onto virgin and grafted prostheses

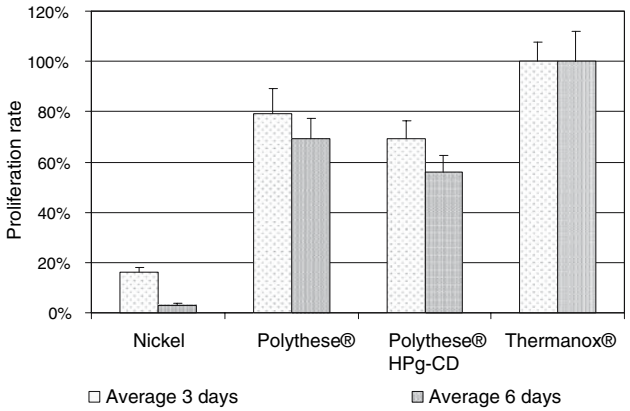


**Fig. 5** Proliferation of HPMEC cells on polythese® and polythese® HP$\gamma$-CD after 3 and 6 days incubation

not optimal on the prostheses (virgin or grafted) whose fibers based structure was not favourable to cell affinity. This indicated that the physical form of the supports was not favourable to cell adhesion and this could explain the decrease of the proliferation rates measured between 3 and 6 days as reported above.

Microbiological assays

Polythese® and Polythese®HP$\gamma$-CD samples previously charged with CFX were dipped in batch systems containing human plasma during 24 h at 37°C. Samples where then periodically withdrawn and laid in a Petri dish in the presence of *S. Epidermidis*. After 24 h of incubation, one could observe (Fig. 7b) a clear zone surrounding each prosthesis aliquot that corresponded to a zone where the bacteria did not proliferate. Therefore, as reported in Fig. 8, the remaining antibacterial activity of the samples was plotted against the time of presence in the plasma batch. One can observe that the inhibition radius which was dependant from the residual antibacterial activity decreased within the 24 h period. This meant that CFX was progressively released from the prostheses samples in the plasma medium and their antibacterial activity consequently decreased. After a stay of 4 h in the batch, we observed no antimicrobial effect at all in the case of Polythese® (Fig. 7a and 8), whereas Polythese®HP$\gamma$-CD exhibited an antimicrobial activity within the whole 24 h test period (Fig. 7b and 8). This prolonged activity of Polythese®HP$\gamma$-CD was undoubtedly due to the CD finishing, and was a consequence of the improved sorption capacity towards CFX mentioned above.

## Conclusion

These investigations showed the possibility of grafting HP$\gamma$-CD onto woven PET vascular prostheses through

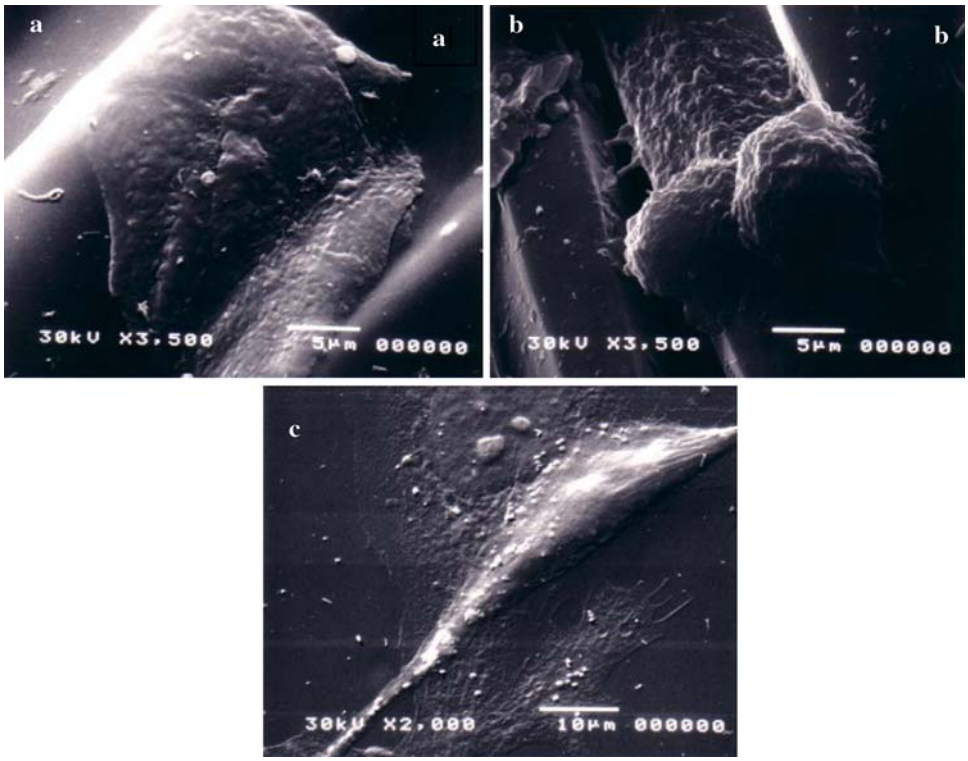


**Fig. 6** Morphology of HPMEC cell on polythese® (a), Polythese® HP$\gamma$-CD (b) and control (c) after 3 days incubation

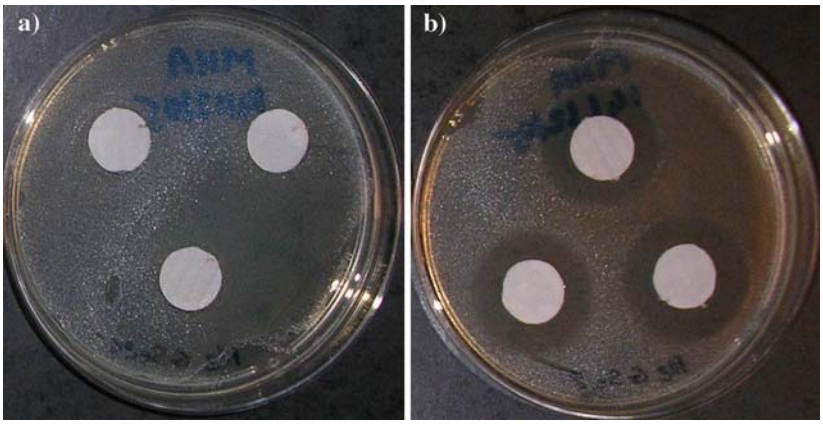


**Fig. 7** microbiological tests. (a) Polythese® (b) Polythese®HP$\gamma$CD Antibacterial activity after 8 hours in plasma batch

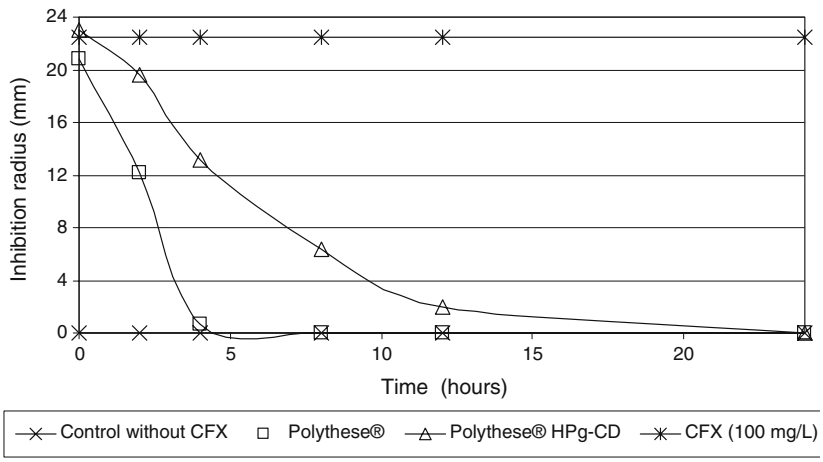


**Fig. 8** Antimicrobial effect of Polythese® and Polythese®HP$\gamma$-CD impregnated with CFX on *S. epidermidis* after different time of incubation in human plasma

a crosslinking reaction that involved the use of citric acid. The weight increase of the fabric and SEM observation confirmed the coating of the surface of the polyester fibers by the polyCTR-HP$\gamma$CD polymer.

Biological investigations showed a lower proliferation rate on Polythese®HP$\gamma$-CD compared to Polythese®. This could not be considered as a toxic effect of the polyCTR-CDHP$\gamma$-coating polymer. Elsewhere, we previously performed viability test that revealed an excellent cytocompatibility of the polyCTR-HP$\gamma$CD polymer even at very high concentration [19]. In fact, fibers present a less favourable surface than films for cell adhesion. So, the proliferation decrease was more a question of physical factors than a problem of toxicity of the CD coating, and this did not affect the biocompatibility of the CD modified prostheses.

In the present paper, the release of the antibiotic was achieved in human plasma that was close to in vivo conditions. As a consequence, results displayed a shorter release time (in the range of 24 h) than what was observed in a previous study and that occurred in pure water [20] (range of several weeks). This reduced release time was due to the difference of ionic force and to the presence of proteins in the blood plasma medium. The interactions of plasma proteins and salts with grafted polyCTR-HP$\gamma$CD played the role of competitor towards sorbed CFX and could explain this shortening of the release time of this antibiotic. Though, whatever the chosen experimental conditions, microbiological data always showed a superior antibacterial activity of the CD grafted prosthesis compared to the virgin prosthesis.

The increased sorption capacity of Polythese®HP$\gamma$-CD and the resulting improved antimicrobial effect was due to a strong enhancement of its sorption capacity, as it could adsorb four times more CFX than Polythese®.

In conclusion, the concept of this drug delivery system based on cyclodextrin grafted woven PET support was efficient in in vitro conditions and is very promising for our goal that was to ameliorate the protection of the patients against post surgery complications due to bacterial proliferation on the vascular grafts.

**Acknowledgements** We are deeply indebted to Laboratoires Perouse (60 173 Ivry-Le-Temple, France), Wacker Fine Chemicals GmbH and Bayer Health Care. We thank Annie Lefèvre for her skilful and expert technical assistance. This work was also financially supported by the Conseil Régional Nord/Pas-de-Calais: ''Federation in Biomaterials Research'' and ARCir ''ASBAMed''.

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