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Comparative study of vascular prostheses coated with polycyclodextrins for controlled ciprofloxacin release

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ABSTRACT

A textile polyester vascular graft was modified with cyclodextrins to obtain a new implant capable of releasing antibiotics (here ciprofloxacin, CFX) over prolonged time periods and thereby reducing the risk of post-operative infections. In this study, we compared samples modified with native and modified cyclodextrins, presenting different cavity sizes (β or γ cyclodextrins) and different substituent groups (hydroxypropyl and methyl). Drug release was measured in water, phosphate buffer pH 7.4 and blood plasma. The inclusion of CFX in the cyclodextrins cavities was observed in solution by two-dimensional ^{1}H NMR spectroscopy and confirmed by ^{1}F NMR measurements. Grafts modification with all cyclodextrins induced an increase of their sorption capacity towards CFX whose extent depended on the nature of the cyclodextrin: a 4-fold and 10-fold increase was observed in the cases of hydroxypropyl cyclodextrins and methylated β-cyclodextrin, respectively. Depending on the type of release medium and nature of CD, different CFX release kinetics were obtained. The discussion highlighted not only the role of the host guest complexation, but also that of the electrostatic interactions that occur between the anionic crosslinks of the cyclodextrins polymers, and CFX that presents a zwitterionic character. The microbiological assessment confirmed sustained CFX release in human plasma and demonstrated antibacterial efficiency of CD modified prostheses against *Staphylococcus aureus* and *Escherichia coli* for at least 24 h (compared to 4 h in the case of virgin grafts).

1. Introduction

Post operative infections in implant surgery are relatively frequent. In the field of vascular surgery, they are declared in approximately 6% of all cases, and induce mortality in 50% of the cases if the infection is localized in aortic position (Bandyk & Esses, 1994; Hennes, Sandmann, Torsello, Kniemeyer, & Grabitz, 1996; O'Brien & Collin, 1992). To overcome this problem, clinicians might use vascular prostheses which locally release antimicrobial agents, such as silver (Batt et al., 2008; Strathmann & Wingender, 2004), single antibiotic drugs (Bergamini et al., 1996; Malassiney, Goëau-Brissonière, Coggia, & Pechère, 1996) or combinations of antibiotic drugs (Javerliat, Goëau-Brissonnière, Sivadon-Tardy, Coggia, & Gaillard, 2007). The efficacy of the latter can significantly differ: for instance rifampicin has been reported to be much more efficient than silver ions in the case of infected prostheses in a dog model: Goeau-Brissonnière (Coggia, Goeau-Brissonnière, Leflon, Nicolas, & Pechère, 2001) and Hernandez-Richter et al. (2003) showed a disappearance of the infection (*Staphylococcus aureus*) when prostheses were impregnated with rifampicin. In contrast, the infection persisted in the case of silver coated prostheses.

The antimicrobial agents are generally loaded onto the vascular prostheses via interactions with collagen, gelatine or other hydrogels, which are commonly used to ensure the blood tightness of the textile structures. However, collagen and gelatine are generally degraded within a few days following implantation. Thus, the maximal release period of the antimicrobial agent is limited and cannot cover the entire healing process.

To overcome this limitation, recently a textile graft, functionalized with cyclodextrins (CDx) has been proposed (Blanchemain et al., 2005). The idea is to cover the textile fibers with a polymeric coating allowing increased drug loading capacities and prolonged periods of controlled drug release. CDx can be used in various

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ways to improve drug administration, in particular to improve the bioavailability of drugs (Loftsson, Jarho, Masson, & Jarvinen, 2005). Many studies have demonstrated the in vivo safety of modified CDx, e.g. hydroxypropyl-β-cyclodextrin (HPβCD) and methyl-β-cyclodextrin (MeβCD) (Frank, Gray, & Weaver, 1976; Irie & Uekama, 1997). In order to immobilize CDx on synthetic textiles (e.g. polyesters and polypropylene) different techniques can be used, such as electronic bombardment (Le Thaut et al., 2000) and plasma treatments (Gawish, Matthews, Wafa, Breidt, & Bourham, 2007). In the present study a "green chemistry process" has been applied, based on a reaction of polyesterification between native or CDx derivates and citric acid (CTR), leading to a crosslinked polymeric network (named polyCTR–CDx) coating the textile fiber (Martel et al., 2006). It has recently been shown that vascular prostheses could be modified with five different types of cyclodextrins (βCD, γCD, HPβCD, HPγCD and MeβCD), (Blanchemain et al., 2007a; Blanchemain et al., 2011) without affecting either their mechanical properties or their safety (Blanchemain et al., 2007b; Jean-Baptiste et al., 2012). Also the importance of the amount of cyclodextrin polymer (PolyCTR-MeβCD) loaded onto the vascular prostheses for the resulting drug release profiles has been studied (Blanchemain et al., 2011).

However, yet it is unclear how the *type* of cyclodextrin affects the release patterns of an incorporated drug. Native CDx significantly differ in size: αCDx exhibits the smallest cavity size (6 glucose units), while γCDx is much larger (8 glucose units). Moreover, the CDx can be chemically modified, e.g. substituted by hydroxypropyl, methyl or other groups. Such modifications might significantly affect the properties of the CDx, in particular their potential to interact with drugs. The aim of this work was to better understand the impact of the type of CDx on the ability to form inclusion complexes with the antibiotic drug ciprofloxacin (CFX) and the consequences on the drug loading capacity and drug release kinetics from CDx modified vascular prostheses.

2. Materials and methods

Woven polyester [Dacron® yarns, poly(ethylene terephthalate), PET] prostheses (Polythese®) were kindly donated by Perouse Médical (Ivry-Le-Temple, France). Cyclodextrins (CDx) used in this study were commercially available, β-cyclodextrin (βCD, Kleptose®), hydroxypropyl-β-cyclodextrin (HPβCD, Kleptose® HPB, MS = 0.62) and 2-*O*-methyl-β-cyclodextrin (MeβCD) (Crysmeb®, DS = 0.50) (Roquette Frères, Lestrem, France); as well as γ-cyclodextrin (γCD, Cavamax® W8) and hydroxypropyl-γ-cyclodextrin (HPγCD, Cavasol® W8, MS = 0.62) (Wacker, Burghausen, Germany). The abbreviation CDx refers to all or some of the CDx mentioned above. Citric acid (CTR) and sodium dihydrogen hypophosphite (NaH_2PO_4, $2H_2O$) were Aldrich Chemicals (Saint Quentin Fallavier, France). Powder of ciprofloxacin hydrochloride (Ciflox®, CFX) was a gift from Bayer Health Care (Leverkusen, Germany).

2.1. Cyclodextrin finishing process and titration of carboxylic groups

The textile functionalization with CDx was based on a *pad-dry-cure* process previously reported (Martel, Morcellet, Ruffin, Ducoroy, & Weltrowski, 2002; Martel, Weltrowski, Ruffin, & Morcellet, 2002; Weltrowski et al., 1999). Samples of woven PET prostheses (1.63 ± 0.12 g) were impregnated and roll-squeezed in an aqueous solution containing CDx, catalyst and CTR, whose composition is reported as *X*/*Y*/*Z*, where *X*, *Y* and *Z* are related to the weight in gram unit of CDx, catalyst and CTR, respectively, dissolved in 100 mL of water. The fixation reaction occurred in a thermo-fixation oven (Minithermo®, Roaches, UK) at 150 °C during 30 min as previously reported (Blanchemain et al., 2008). After this treatment, all prostheses were thoroughly washed in a Soxhlet extractor with hot water in order to remove the unreacted products. All samples were preliminarily dried at 104 °C and cooled down to room temperature in a desiccator during 30 min. The degree of functionalization (DF, unit wt%) is reported as the weight gain of the samples according to the following equation:

$$\mathrm{DF} = \frac{m_f - m_i}{m_i} \times 100 \quad (1)$$

where m_i and m_f denote the sample weight before and after treatment, measured with a precision balance ($\pm 4 \times 10^{-4}$ g). In the present study, the DF of the modified prostheses with CDx (PET-βCD, PET-HPβCD, PET-γ-CD and PET-HPγCD) was 18.0 ± 0.5 wt%. Issued from the polycondensation between CTR and CDx, the resulting coating polymer also carried residual free carboxylic functions (Ducoroy, Bacquet, Martel, & Morcellet, 2008; Ducoroy, Martel, Bacquet, & Morcellet, 2007). The amount of free carboxylic groups on the vascular prostheses was determined using a calcium acetate titration method (USP, 1995). Five hundred mg of vascular prosthesis was placed in 50 mL of a calcium acetate solution (2 wt%) during 30 min under stirring (200 rpm). The mixture was titrated using a 0.05 N NaOH solution (indicator = phenolphthalein) (Kumar & Yang, 2002). The amount of free carboxylic functions was expressed in mmol of COOH groups per gram of prosthesis and calculated as follows:

$$\text{COOH functions/prosthesis (nmol/g)} = \frac{N \times V_e}{\text{sample weight (g)}} \quad (2)$$

where N and V_e denote the molarity of the NaOH solution (nmol/L) and the equivalent volume (L), respectively.

2.2. NMR studies

^{1}H spectra were recorded on a Ultrashield 9.4 T (400 MHz) Avance II, BRUKER spectrometer. The complexation between CDx and CFX was studied by application of the continuous variation method reported by Job (Connors, 1987; Gil & Oliveira, 1990), which consisted of preparing 10^{-2} mol/L stock solutions of CFX and γCD or HPγCD solutions in D_2O. Aliquots of both solutions were placed in 5 mm diameter NMR tubes at different volume ratios varying from 0/10 to 10/0 (CD/CFX) and mixed by sonication during 20 min. The total volume in the tubes was kept constant at 600 μL. The *Job plot* obtained allowed determining the stoichiometry of the inclusion complex. The geometry and orientation of the guest molecule in the CDx cavity were determined from the two-dimensional Roesy experiments (Hwang & Shaka, 1992) displaying the dipolar interactions between the host and the guest molecules in the supramolecular assembly. The pulse for ROESY spinlock was 600 ms. Fluor NMR was also assessed to investigate the interactions between the fluorinated quinolone and the CDx cavity. Inclusions models were obtained from ACD/HNMR predictor and Raswin.

2.3. Drug sorption studies

Modified and virgin prostheses were cut into 10 mm diameter disks (27.2 ± 1.8 mg) and dipped into a CFX solution (2 g/L) during 4 h. The total amount of loaded drug was determined after desorption of the prostheses in a 0.05 M sodium hydroxide solution (4 h at 37 °C). The latter treatment leads to a complete hydrolysis of the CD polymer coating and the complete release of CFX from the prostheses. The CFX concentration in the supernatant was determined at 271 nm using a UV-spectrophotometer (UV-1800, Shimadzu, Marne La Vallée, France). Five replicates were performed.

2.4. Drug release measurements

Drug loaded prostheses (27.5 ± 1.6 mg) were placed into vials filled with 10 mL of purified water, phosphate buffer pH 7.4 (United States Pharmacopeia 32) or human plasma (Etablissement Français du sang, Lille, France) in a horizontal shaker (80 rpm, 37 °C) (Innova® 40, New Brunswick Scientific, Le Pecq, France). At pre-determined time points, the supernatant solution was completely renewed and the drug content in the withdrawn bulk fluid determined by UV-spectrophotometry (UV-1800 Shimadzu) at λ = 271 and 280 nm in phosphate buffer and water respectively. All experiments were conducted in triplicate.

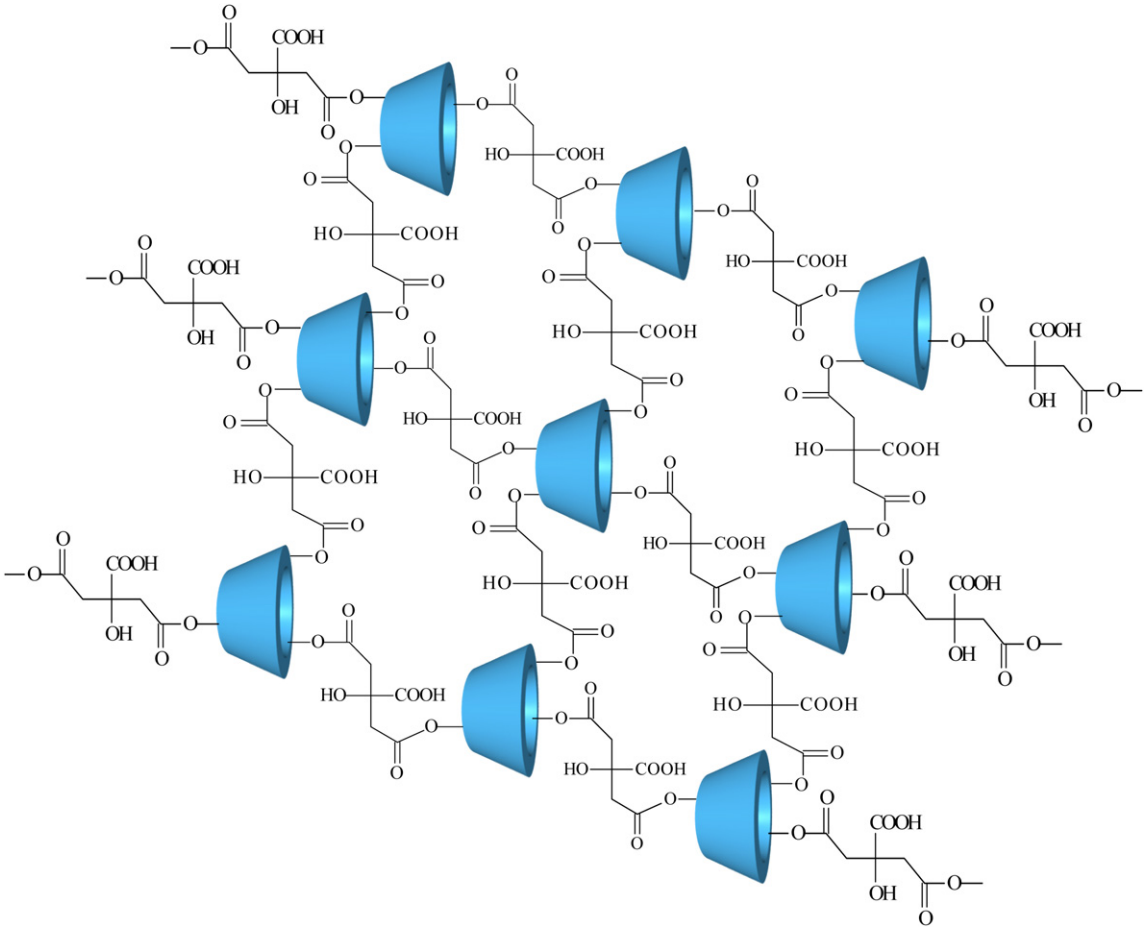
2.5. Microbiological analysis

Microbiological tests were performed according to the standardized Kirby–Bauer method (Scott & Higham, 2003). Drug loaded prosthesis samples (disc-shaped, 6 mm in diameter) were incubated in 1 mL human plasma (Etablissement Français du Sang, Lille, France) for up to 24 h in a horizontal shaker at 80 rpm, 37 °C. At predetermined time points, samples were removed and placed into plates containing Mueller Hinton agar seeded with *S. aureus* (CIP224, collection strain) or *Escherichia coli* (strain L70A4 recently isolated from a clinical sample), while the human plasma was collected in a 5 mL Eppendorf® vessel for HPLC analysis in order to assess the drug amount released from the sample into the supernatant. After 24 h incubation at 37 °C, the radius of the circular zone with growth inhibition was measured around the disc samples. These values are plotted as a function of the contact time with the human plasma.

3. Results

3.1. Titration of the free carboxylic groups

Functionalization of the prostheses occurred by the in situ reaction between citric acid and cyclodextrins, yielding a polymer (polyCTR–CDx) whose structure was based on cyclodextrin moieties linked to each other via esterified citric acid residues carrying free carboxylic groups (see Scheme 1). As a consequence, the titration of these carboxylic functions can be used to characterize the coating polymer (Ducoroy et al., 2008; Ducoroy et al., 2007). Fig. 1 shows the concentration of free carboxylic groups (in mmol/g) determined with vascular prostheses coated with the different types of CDx. In all cases the degree of functionalization was 18 ± 0.5 wt%. As it can be seen, PET samples functionalized with *native* cyclodextrins (PET-γCD and PET-βCD) presented 325 and 341 nmol COOH groups per gram, thus, about 35% more free carboxylic groups than prostheses modified with hydroxypropyl cyclodextrins (PET-HPγCD and PET-HPβCD, containing 216 and 230 nmol COOH groups per g). MeβCD finished prostheses (PET-MeβCD) showed intermediate free carboxylic group concentrations. The difference in COOH content can be attributed to different conversion rates of the acidic groups of CTR into ester groups when the crosslinking agent came in contact with native or modified CDx. These results indicate that hydroxypropyl CDx more readily undergo esterification with CTR than do native CDx



Scheme 1. Structure of the coating polymer consisting of CDx units, which are cross-linked with CTR via ester bounds, and bearing residual, free carboxylic functions.

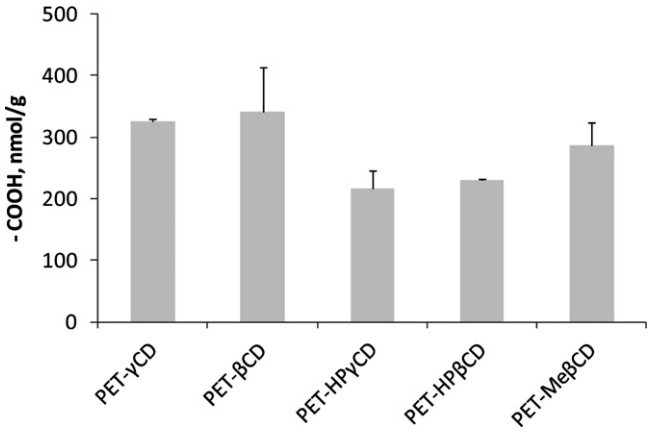


Fig. 1. Concentration of free carboxylic groups in the different functionalized vascular prostheses.

and therefore the former present a lower amount of residual free acidic functions. These results confirm previous reports demonstrating that hydroxypropyl CDx exhibit higher reactivity than native CDx: their fixation onto polyester supports occurred at lower temperatures and more rapidly (Blanchemain et al., 2007a; Martel, Morcellet, et al., 2002).

3.2. NMR complexation studies

The formation of an inclusion complex between βCD, MeβCD, and HPβCD and ciprofloxacin has been previously demonstrated (Blanchemain et al., 2011). In all cases, 1/1 complexes were formed by inclusion of the piperazinyl group of these CDx. In the present study, we additionally investigated the complexation of CFX by γCD and HPγCD. The Job plot shown in Fig. 2 indicates a maximum centred at $r = 0.5$ for the γCD/CFX complex, thus, 1:1 complex is formed. The same result was obtained in the case of HPγCD (data not shown). Furthermore, the ROESY spectra of CFX/CDx stoichiometric mixtures displayed in Fig. 3a and b show correlation tasks that reveal dipolar interactions between H2 and H6 of the piperazinyl group of CFX with H3 of both, HPγCD and γCD, respectively. Neither protons H5 of both CDx nor protons H3 and H5 of CFX displayed any correlation tasks. This led us to propose the first inclusion model presented in Fig. 4a, where the piperazinyl group of CFX penetrates the γCD or HPγCD cavity.

In addition, it can be seen in Fig. 3c that H3 and H5, the inner protons of the cavity of γCD also displayed dipolar interactions with

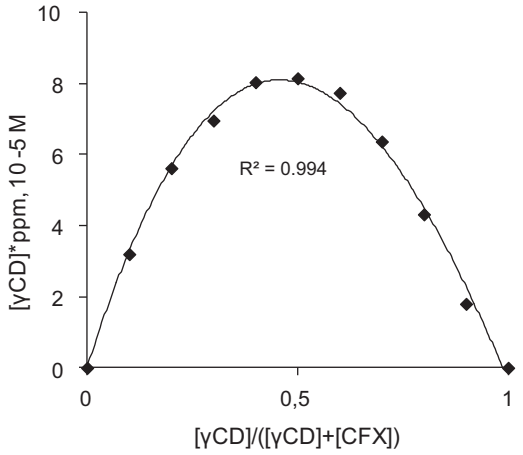


Fig. 2. Continuous variation plot (Job plot) of the γCD/CFX system.

proton H9 present on the quinolone part of CFX, and with H13 situated in an intermediate position between the carboxylic group and the tertiary amine of CFX bearing the cyclopropyl group. This indicates that CFX could also be included in γCD via its side terminated by the carboxylic acid as shown in Fig. 4b. Hence, two different 1:1 complex geometries may coexist in the case of γCD/CFX mixtures.

The fluor NMR spectra reported in Fig. 5, show the shift of the signal after addition of βCD in stoichiometric ratio. This variation revealed a change of the environment of the fluorine atom which is due to the inclusion of the carbon ring of the fluoroquinolone group of CFX inside the cavity. As it can be seen in Fig. 5, a shift of the fluorine signal varying from 32 to 38 Hz was observed with all the investigated CDx/CFX complexes, except for γCD/CFX. In the latter case a shift of only 12 Hz was observed. This result confirms the above mentioned hypothesis that two types of γCD/CFX complexes co-exist. Two sites of the drug can be included in the cavity: the piperazinyl and the fluoroquinolone group on one hand, and the heterocyclic ring of the quinolone on the other hand.

3.3. Drug loading onto the prostheses

Upon exposure of PET-βCD, PET-γCD, PET-HPβCD and PET-HPγCD samples to an aqueous CFX solution (2 g/L) for 4 h, the total amount of CFX loaded onto the device was determined by UV spectrophotometry at 270 nm (Fig. 6). Generally, the CFX loading onto native and methylated CDx finished PET prostheses was much higher than that onto control samples, and onto γCD and HPγCD finished PET prostheses. A 9-10 fold increase of the CFX loading was observed comparing PET and PET functionalized with native CDx. This factor decreased to 3–4 fold in the case of hydroxypropyl CDx. As discussed above, the presence of the hydroxypropyl groups involves a higher crosslinking degree of the polymeric network (due to their higher reactivity towards citric acid). At the same time, the presence of the hydroxypropyl groups on the primary and the secondary rims of the macrocylic structure of CDx may also favor intramolecular reactions with citric acid (as illustrated in Scheme 2), resulting in the presence of a bulky group limiting the accessibility of the CD cavity for CFX.

3.4. Ciprofloxacin release

Fig. 7 illustrates the absolute (left hand side) and relative (right hand side) CFX release rates from virgin and PET-βCD, PET-γCD, PET-HPβCD and PET-HPγCD modified prostheses in water, phosphate buffer pH 7.4 and human plasma, respectively (note the different scaling of the *x*-axes). Clearly, drug release from virgin prostheses occurred within a few minutes in all media. In contrast, modified prostheses liberated CFX within 50 to more than 80 days in pure water. Though, CFX release was less sustained in phosphate buffer pH 7.4 and plasma as it occurred within a few hours. The influence of the nature of the immobilized cyclodextrin on the sustained release effect depended on the composition of the release medium. On the one hand, the best results were obtained with HPβCD system that released 80% of its loaded drug within 75 days, against 3 days for MeβCD and γCD in PBS. In blood plasma, the later supports released 80% of their loaded drug within and 8.5 and 6 days, respectively. So the best system was based on HPbCD in water, and MebCD and gCD in natural and synthetic physiological media.

3.5. Microbiological tests

The antibacterial efficiency of CFX loaded PET prostheses functionalized with CDx was compared to the PET prostheses as control. The antibacterial activity of the prostheses functionalized with CDx is prolonged (at least 24 h), whereas the activity of the PET

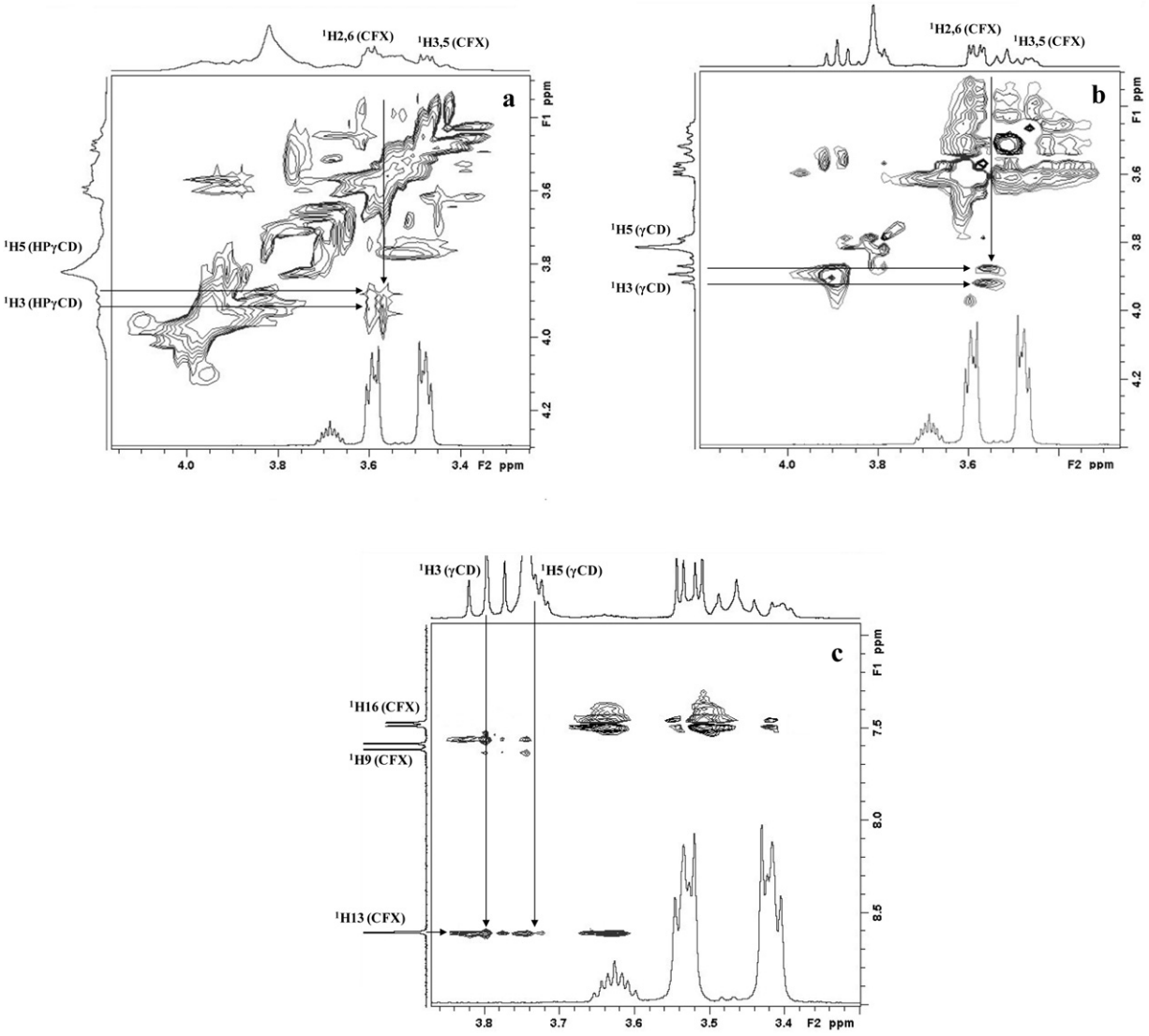


Fig. 3. ROESY spectrum of the 1:1 HPγCD/CFX or 1:1 γCD/CFX complex (a) and the 1:1 γCD/CFX complex (b and c) in D_2O (10^{-2} M).

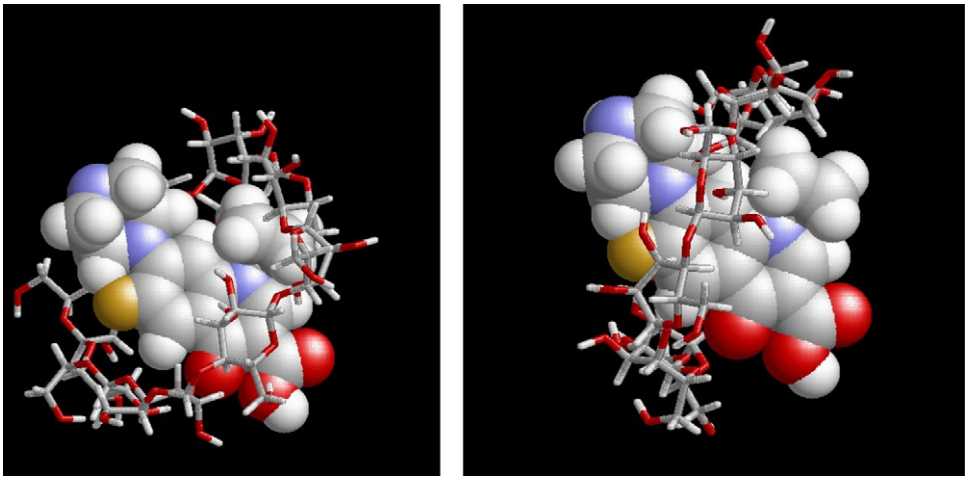


Fig. 4. Schematic representation showing the structure of the HPγCD/CFX and γCD/CFX complex via the piperazinyl group (a) and γCD/CFX complex via the carboxylic group (b).

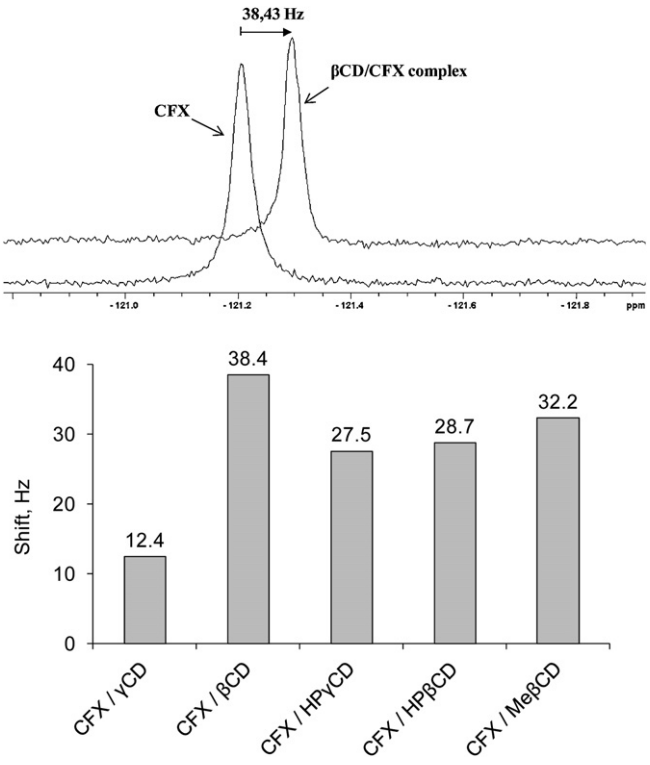


Fig. 5. Chemical shift of the fluorine peak of CFX upon complexation with cyclodextrin (1:1 CFX/CD complex, 10^{-2} M).

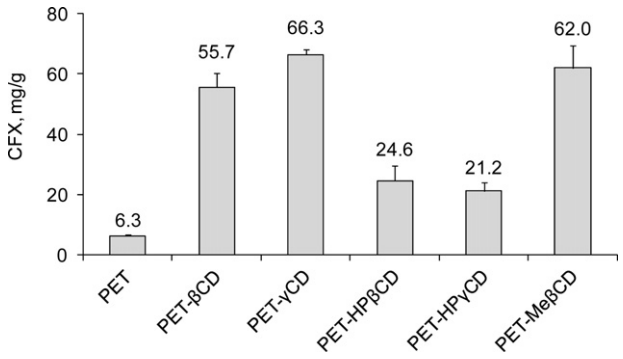


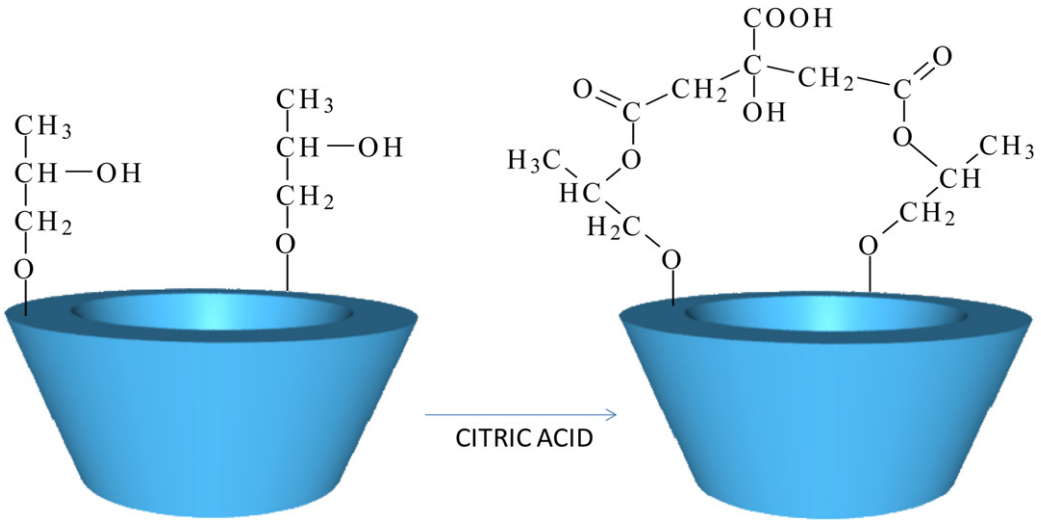
Fig. 6. Amount of CFX loaded onto virgin and CD treated prostheses expressed in mg/g; sorption time = 4 h.

prostheses was 4–5 h at best (Fig. 8). On the two bacterial stains, no significant difference was observed between the four CDx at day 1. The CFX loaded PET prosthesis was less efficient on *S. aureus* (Fig. 8a) than on *E. coli* (Fig. 8b) due to the specific spectrum of activity of the chosen antibiotic.

4. Discussion

It was recently shown that vascular prostheses functionalized with CDx have the capacity to release a drug in a controlled manner over prolonged periods of time (Blanchemain et al., 2008). The release kinetics of the drug depends on several factors, in particular the quantity of CDx polymer fixed onto the prosthesis (Blanchemain et al., 2011). The aim of the present study is to investigate the impact of the nature of the CDx chosen among native, methylated and hydroxypropyl derivates. The size of the CDx cavities (βCD, γCD), the nature of their substituents (hydroxypropyl or methyl groups) and their degree of substitution can be expected to affect the affinity of the drug to the polyCTR–CDx fixed onto the prostheses as well as the resulting drug release kinetics.

CFX was selected in this study because of its widespread use in the field of the vascular surgery and also for its affinity to CDx. A 1:1 inclusion complex between CFX and βCD and HPβCD has been reported by Jianbin (Jianbin, Liang, Hao, & Dongpin, 2002; Jianbin, Dongpin, Li, & Huang, 2004) and we recently showed the same type of inclusion with MeβCD. The present study additionally demonstrates identical inclusion complexes of the 1:1 type between CFX and the following CDx: βCD, HPβCD, MeβCD and HPγCD. In these cases, all CDx encapsulate the piperazinyl group and the fluorinated ring of the quinolone of CFX. In contrast, in the case of γCD, the complex (being also of the 1:1 type as displayed by the Job plot), ROESY NMR measurements indicate that also the part of the quinolone bearing the carboxylic acid function can enter the CDx cavity. The results obtained by the ROESY sequence were confirmed by the observation of the fluorine NMR spectrum showing a weaker shift of the fluorine signal of CFX in the presence of γCD compared to the four other CDx. This can be explained by the larger size of the γCD cavity compared to βCD, that should offer more possibilities for the fitting with the guest molecule. Indeed, NMR data showed that the presence of the hydroxypropyl substituents of HPγCD prevented



Scheme 2. Intramolecular reaction between two hydroxypropyl groups of CDx and citric acid yielding a "self bridged" cyclodextrin.

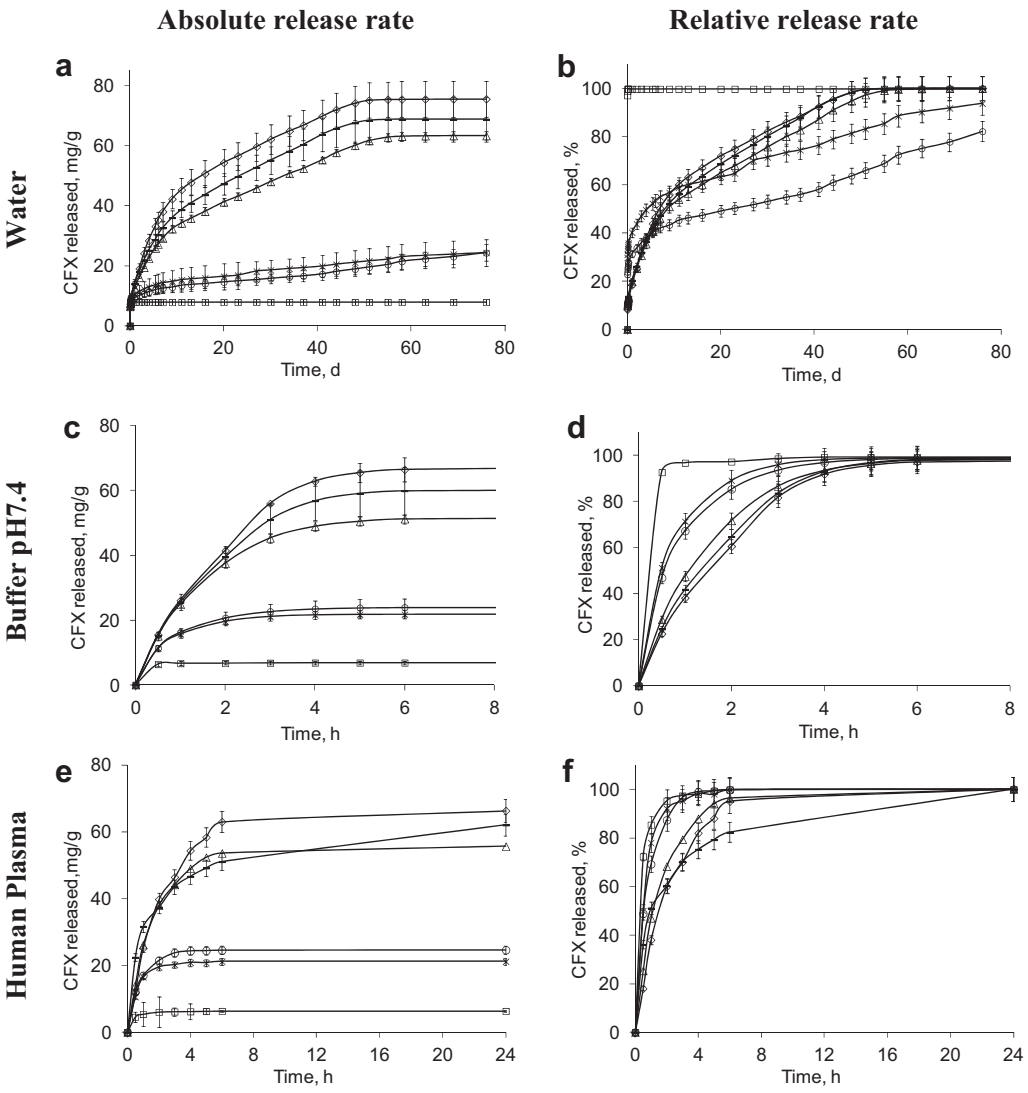


Fig. 7. Release kinetics of CFX in purified water (a and b), phosphate buffer pH 7.4 (c and d) and human plasma (e and f) from virgin and CD finished prostheses. □, virgin PET; △, βCD; ◊, γCD; ○, HPβCD, ×, HPγCD, –, MeβCD.

the encapsulation by the carboxylic end, but allowed the inclusion through the piperazinyl group, like βCD and its derivates do.

The titration of the free carboxylic functions present on the samples displayed higher contents of COOH groups on prostheses treated with native and methylated CDx than prostheses treated with βCD and HPγCD. As all samples have been treated using the same curing conditions (160 °C during 30 min), these results demonstrate that CDx present a different reactivity towards citric acid. In fact, CDx substituted with hydroxypropyl groups are more reactive than their parent native cyclodextrins. This is consistent with literature reports (Martel, Weltrowski, et al., 2002). It has previously been demonstrated that hydroxypropyl CDx can be fixed onto polyester and polyamide (El Ghoul et al., 2007) textile supports at temperatures and time of thermofixation inferior to those applied to native CDx. For these reasons the first studies that we reported on vascular prostheses involved hydroxypropyl cyclodextrins (Blanchemain et al., 2007a; Blanchemain et al., 2008; Jean-Baptiste et al., 2012; Martel, Morcellet, et al., 2002), due to softer reaction conditions lowering the risks of thermal degradation of the polyester.

The origin of the higher reactivity of hydroxypropyl CDx is probably the better accessibility of the hydroxypropyl substituent towards citric acid. As a consequence, the reaction of esterification is more complete and polyCTR-HPβCD and polyCTR-HPγCD present a reduced amount of free COOH groups compared to native or methylated cyclodextrin based polymers. Another consequence of this feature is that both types of polymers should present a higher crosslinking degree, due to a higher extension of the esterification, therefore the polymer network is probably more dense and the access to the cavities probably reduced. This latter phenomenon can also be emphasized by the possible intramolecular reaction between two hydroxyl groups of the hydroxypropyl substituents and citric acid resulting in a *intramolecular bridge* that could hinder the cavity access to the host molecule (Scheme 2).

The acidic functions mentioned above may display ionic interactions with drugs carrying basic amino groups and play a role in the adsorption release properties of the supports. This is especially the case of CFX which carries a piperazinyl and a quinolone group, that present one secondary and two tertiary amino groups which are in their salt form in the conditions of the release experiments. Consequently, the loading of such amino containing drugs onto the prostheses can be expected to occur not only through *host–guest* complexation into the CD cavities, but also via acid–base interactions.

Indeed, this was confirmed by the results of the loading capacity tests that displayed a superior capacity of the native CDx and

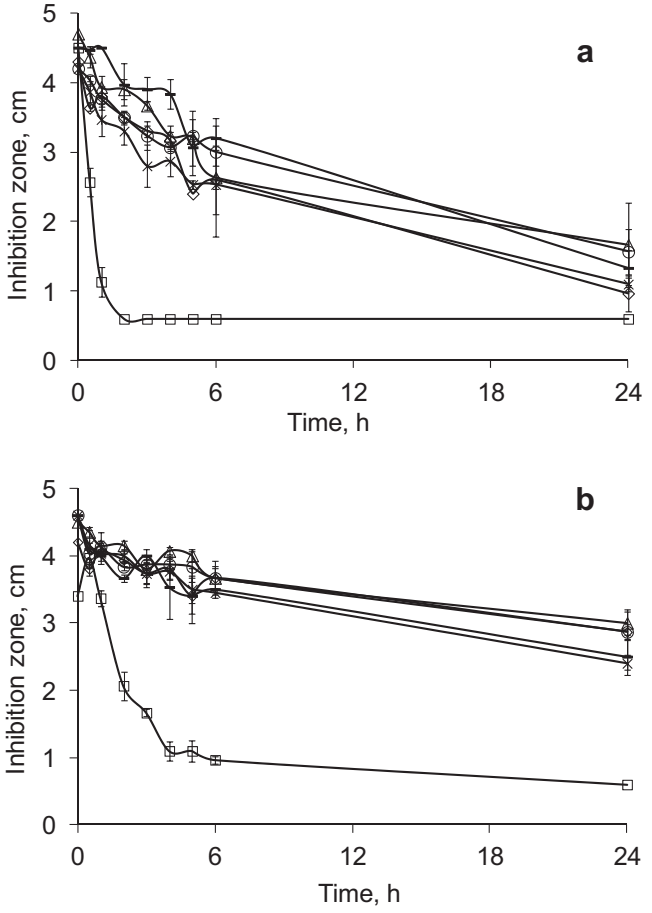


Fig. 8. Antimicrobial activity of virgin and CD finished prostheses loaded with CFX against: (a) *Staphylococcus aureus*, (b) *Escherichia coli*. Evolution of the size of the inhibition zone as a function of the contact time with plasma. □, virgin PET; △, βCD; ◇, γCD; ○, HPβCD; ×, HPγCD; –, MeβCD.

MeβCD functionalized prostheses despite the same functionalization degree of all prostheses (18 wt%) meaning that samples carried the same order of magnitude of CDx content. So the major difference between all samples stood in their COOH groups content, which can explain the important differences in the amounts of CFX loaded onto prostheses modified with hydroxypropyl CDx and the other prostheses.

A large influence of the nature of the release medium on the kinetics of the release of the antibiotic was observed. The presence of ions in phosphate buffer pH 7.4 and of ions and organic compounds in plasma may play a competitor role towards the CFX/CDx inclusion complexes and explain why the total release of the drug occurred within 50–80 days in water compared to only a few hours in phosphate buffer pH 7.4 and plasma. This highlights the importance of the ionic phenomena in the interactions between CFX and polyCTR–CDx. In addition, due to the zwiterionic character of ciprofloxacin the prosthese–drug interactions, also depend on the pH of the medium. As a matter of fact, at acidic pH, both piperazinyl and carboxylic functions of CFX are protonated, yielding a cationic character to the drug. On the contrary, at pH 7.4 applied in the study, chosen for mimicking the physiologic conditions, the carboxylic groups are transformed into carboxylate groups, and CFX presents an anionic character (Hernández-Borrel & Montero, 1997). As a consequence, electrostatic repulsion effects occur between CFX and the carboxylate functions carried by the crosslinks of the CDx polymer and this should contribute to the faster release observed in PBS and blood plasma, compared to pure water medium. This phenomenon has been recently underlined in a paper dealing with cyclodextrin–agar hydrogels for ciprofloxacin delivery (Blanco-Fernandez, Lopez-Viota, Concheiro, & Alvarez-Lorenzo, 2011).

The biological evaluation shows a prolonged antibacterial activity of the five functionalized prosthesis (for more than 24 h) compared to the virgin prosthesis loaded with CFX (4 h) on two strains (*S. aureus*, *E. coli*). However, no significant difference between the different functionalized prostheses was observed. The antibacterial activity persists over 24 h, while the quantity of released CFX fell below the detection limit of the applied analytical method. This clearly indicates that a sufficient quantity of CFX remains on the prosthesis, efficient against the bacteria, even after 24 h. Thus, the proposed functionalized prostheses are likely to offer a protection against infections not only during the first hours, but during the first day after surgery when the post operative infectious risk is highest.

5. Conclusion

In this work, five different cyclodextrins were fixed onto a vascular graft for the sustained release of ciprofloxacin. The size of the CDx cavities (βCD, γCD), the nature of its substituents (hydroxypropyl or methyl groups) was the first topic of the discussion. Firstly, we evidenced through advanced NMR studies the complexation of CFX with βCD, γCD and HPγCD. Then we investigated the influence of the nature of the immobilized CDx on the sorption capacity of the supports. The best results were obtained with βCD, γCD and MeβCD, compared to HPβCD and HPγCD. Release tests showed the most marked sustained release effect with HPβCD in pure water, while MeβCD and γCD gave the best results in synthetic and natural physiological media. Therefore the drug–prostheses interactions were not only discussed in terms of host–guest complexation, but also in terms of ionic and acid–base interactions due to the presence of carboxylate functions in the structure of the CDx polymer.

The zwiterionic nature of CFX was identified to be at the origin of all those phenomena. Importantly, this paper evidenced that the nature of the cyclodextrin was a key parameter in the process of functionalization of a medical device for the control of the sorption and release of a drug. In particular, the native or methylated βCD will be good candidates for the vascular application especially thanks to the higher sorption capacity, in order to limit post operative infections or to treat a resumption of vascular graft infection. Nevertheless, through in vivo tests it will be necessary to take into account the potential local toxicity effects due to the high quantity of loaded CFX on the prosthesis.

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