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Mussel Inspired Coating of a Biocompatible Cyclodextrin Based Polymer onto CoCr Vascular Stents

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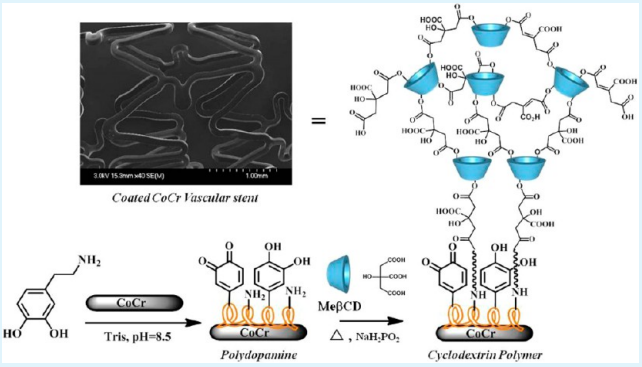
Supporting Information

ABSTRACT: During the past decade, drug-eluting stents (DES) have been widely used for the treatment of occlusive coronary artery diseases. They are supposed to reduce the incidence of early in-stent restenosis by the elution of highly hydrophobic antiproliferative drugs. Nevertheless, the absence of long-term activity of these devices is responsible for late acute thrombosis probably due to the delayed re-endothelialization of the arterial wall over the bare metallic stent struts. Thus, a new generation of DES with a sustained release of therapeutic agents is required to improve long-term results of these devices. In this article, we report an original functionalization of CoCr vascular devices with a hydrophilic, biocompatible and biodegradable cyclodextrins based polymer which acts as a reservoir for lipophilic drugs allowing the sustained release of antiproliferative drugs. In this setting, polydopamine (PDA), a strong adhesive biopolymer, was applied as a first coating layer onto the surface of the metallic CoCr device in order to promote the strong anchorage of a cyclodextrin polymer. This polymer was generated "in situ" from the methylated cyclodextrins and citric acid as a cross-linking agent through a polycondensation reaction. After optimization of the grafting process, the amount of cyclodextrin polymer coated onto the CoCr device was quantified by colorimetric titrations and the resulting film was characterized by scanning electron microscopy (SEM) investigations. The cytocompatibility of the resulting coated film was assessed by cell proliferation and vitality tests. Finally, the ability of this coated device to act as a drug-eluting system was evaluated with paclitaxel, a strong hydrophobic antiproliferative drug, a reference drug used in current vascular drug-eluting stents.



KEYWORDS: *drug-eluting stent, restenosis, polydopamine, cyclodextrin polymer, biocompatibility, antiproliferative drug*

INTRODUCTION

Bare metal stents prevent immediate artery recoil and reduce the restenosis rate after balloon angioplasty.[1] Their efficacy is hampered by smooth muscle cells (SMCs) migration/proliferation, which results in neointimal hyperplasia. Drug-eluting stents based on the local elution of highly hydrophobic antiproliferative drugs such as paclitaxel, sirolimus or everolimus have emerged to limit this pathophysiological phenomenon but they are associated with high rates of late acute in-stent thrombosis. This latter is probably promoted by the delayed re-endothelialization of the arterial wall associated with the presence of a nonabsorbable and proinflammatory polymer, which elutes the antiproliferative drug as the occurrence of this latter complication was reduced with bare metal stents. The nature of the surface deployed against the arterial wall is of prime importance to study cell reactions and it is now well accepted that bioabsorbable and non-proinflammatory molecules must be applied in that field. Recent publications promote the development of an entire bioresorbable scaffold that is expected to replace the classical metallic structure in the near future.[2−4] However, the mechanical properties of the bioresorbable scaffold cannot be compared with CoCr or stainless steel conventional vascular stents. An alternative strategy consists in the immobilization of specific

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biocompatible coatings allowing the generation of nitric oxide which plays several physiological roles such as the inhibition of platelets aggregation, the reduction of SMCs proliferation while promoting endothelial cell (ECs) proliferation.[5,6]

The coating of a synthetic hydrophilic biocompatible polymer onto the metallic surface acting as control releasing layer would be an appealing choice for prolonged biocompatibility but it appears challenging to load any lipophilic prohealing drugs within any hydrophilic biopolymer due to their low affinity and, therefore, a high storage capacity for such systems cannot be expected.

Cyclodextrins, according to the hydrophobic character of their cavities,[7−10] are well-known to form reversible inclusion complexes with many hydrophobic molecules and are widely used for their encapsulation properties as drug carriers.[11−13] Recently, Martel et al. have developed an original approach consisting of the grafting of a cyclodextrin polymer onto various biomaterials such as polyethylene terephthalate (PET) vascular textile,[14] porous hydroxyapatite[15] and visceral meshes.[16] In these functionalized materials, the cyclodextrin polymer attached to the support via physical interactions acts as a reservoir for the sustained release of one or more bioactive molecules. In a recent publication, Taha et al.[17] reported an original approach to modify an hydroxyapatite coated orthopedic hip implant with a cyclodextrin polymer acting as drug-delivery system. In that latter case, the titanium substrate was coated with a plasma-sprayed hydroxyapatite layer which exhibits high hydrophobicity and porosity properties promoting the anchorage of the cyclodextrin polymer. The main challenge to transpose this approach to the modification of CoCr biomaterials is to anchor the cyclodextrin polymer strongly onto the mirror polished (nonporous) CoCr surface.

In 2007, inspired by the composition of the adhesive proteins of mussels, Lee et al.[18] developed a biomimetic approach for the functionalization of a wide range of material including biomaterials such 316 L, stainless steel, titanium and CoCr with an adhesive polydopamine (PDA) layer.[18,19] Very recently, Huang et al.[20] have demonstrated that PDA films provide a noncytotoxic and hemocompatible platform for vascular devices. These authors have also demonstrated that PDA promotes endothelial cell (ECs) proliferation while reducing SMCs proliferation.[20,21]

In this work, we propose to functionalize CoCr vascular metallic stents by using an original surface modification approach combining the strong adhesive properties of the PDA with the drug storage capacities of cyclodextrin polymers allowing the sustained release of prohealing arterial drugs.

EXPERIMENTAL SECTION

Materials and Methods. All reagents were purchased from Sigma-Aldrich (St. Louis, MO) and were used as received except for 2-O-methyl- β-cyclodextrin (MeβCD, Crysmeb, DS = 0.50) which was provided by Roquette Frères (Lestrem, France). Ultrapure water (18.2 MΩ, Millipore Milli-Q system, Merck KGaA, Germany) was used for all the experiments. Paclitaxel was purchased from INRESA (Bartenheim, France) and docetaxel from Sigma Aldrich. Water-soluble MeβCD polymer (M_n = 25 kDa measured by size exclusion chromatography (multiple angle laser light scattering)) was synthesized in order to investigate the phase solubility diagram of paclitaxel (PTX) in aqueous medium. This polymer was obtained by polycondensation between citric acid and methylated cyclodextrins (PolyCTR-MeβCD) as described previously.[22,23]

CoCr disks were cut into a cobalt−chromium rod (⌀14.5 mm) according to ISO standards 5832-12 (Co 66.00%, Cr 27.34%, Mo 5.19%, Mn 0.56%, Si 0.39%) bought from Böhler-Edelstahl, Germany with a thickness of 3 mm each.

For all experiments (i.e., quantifications, kinetics and biological evaluations), the measurements were repeated three times to obtain an average value.

Preparation of CoCr Samples. CoCr disks were polished with an automatic polishing device (PHOENIX 4000 (Sample Preparation System), Buehler, Germany) after 4 cycles of 7 min each (using subsequently 600, 1200, 2400 and Supra5 sand papers, from PRESI, France). Disks were cleaned by a 5 min sonication (44 kHz, Blackstone-NEY Ultrasonics, Jamestown, NY) in acetone, water and ethanol.

The chemical oxidation of CoCr plates was performed using "piranha" solution mixture with a 70/30 v/v ratio between sulfuric acid (H_2SO_4, 97 wt %) and hydrogen peroxide (H_2O_2, 36 wt %).[24] Disk samples were immerged for 60 h at room temperature under extractor hood. The disks were then cleaned by sonication in water (30 mn, 4 times).

Polydopamine (PDA) Coating Layer. The cleaned CoCr disks were placed in a pillbox containing 5 mL of dopamine (2 mg/mL) solution in a 10 mM Trizma buffer adjusted to pH 8.5.[25] The reaction was performed at room temperature (RT), under 400 rpm for 16h. The PDA treated CoCr plates were rinsed with deionized water, dried at RT and placed in a clean ventilated oven for a thermal treatment at four different temperatures (150 °C, 160 °C, 170 or 180 °C) for 1 h.

Cyclodextrin Coating Layer. Disk samples previously functionalized by PDA were immerged in a citric acid (CTR)/$Na_2H_2PO_2$/MeβCD solution with a 10/3/10 ratio (in g per 100 mL of water) for 10 min at 37 °C (80 rpm) and were dried in a ventilated oven for 30 min at 90 °C. Sodium hypophosphite ($Na_2H_2PO_2$) was used as a catalyst and CTR as a cross-linker. Thereafter, the polycondensation of MeβCD and CTR was performed by curing at three different temperatures: 140, 150, and 160 °C and at four different durations: 20, 30, 45, and 60 min.[26] This process resulted in the fixation of a cyclodextrin polymer on PDA layer denoted in this study as polyCD (PCD).

Colorimetric Titrations. The grafting process was optimized to obtain the best compromise between the functionalization rate of the surface by the cyclodextrin polymer, the maximal drug amount loaded onto CoCr disks, and its subsequent longest release time.

The quantification of grafted MeβCD polymer onto the metallic disk surfaces was evaluated with Toluidine Blue Ortho (TBO) by UV−visible titration (UV-1800 Shimadzu). On the one hand, the carboxylic acid functions of the MeβCD polymer interact with the cationic site of the TBO molecule through an ion exchange mechanism. On the other hand, TBO is also entrapped into the MeβCD cavities due to its polycyclic and aromatic structure.[27] TBO is an aromatic blue dye whose quantification is determined by visible spectrophotometry at 641 nm after standardization.[27,28]

In this study, TBO titrations were used (i) to quantify the amount of cyclodextrin polymer grafted onto the CoCr surfaces, (ii) to optimize the parameters involved in the cyclodextrin polymer grafting process, and (iii) as a drug model to evaluate the sustained kinetic release properties of the modified CoCr implants.

A 6×10^{-4} M TBO solution was prepared in water and adjusted to pH 10 with a 0.1 M NaOH solution. Disk samples were immersed in 20 mL of the TBO solution at 37 °C. After 4 h of impregnation, the excess of TBO loaded and noncomplexed onto the metallic surface was removed twice in 10 mL of a 10^{-4} M NaOH solution. The desorption of complexed TBO from the treated surface was performed in 20 mL of acetic acid for 20 min (acetic acid, 50%; peak absorbance, λ = 641 nm). The kinetic release of TBO of functionalized disk samples over time at subsequent intervals was assessed in 10 mL of phosphate buffered saline solution (PBS, pH 7.4) (peak absorbance, λ = 288 nm). The results were expressed as a percentage of the accumulated quantity of released TBO at each predetermined time point to that of the total amount of adsorbed TBO. The degradation of the coating was also investigated in a culture medium (Endothelial Cell Growth Medium MV (Promocell GmbH, Heidelberg, Germany)) using the TBO method mentioned above; after 4, 8, 24, 72, and 96 h

of contact with both media, the disk samples were rinsed with pure water, then immersed in 20 mL of the TBO solution for 4 h at 37 °C, rinsed twice in 10 mL of a 10^{-4} M NaOH solution, and desorbed with 20 mL of 50% acetic acid as exposed above. The quantification of the remaining MeβCD polymer onto disks was thus achieved.

Analytical Techniques. Spectroscopic ellipsometry (SE) measurements were performed at room temperature using a phase-modulated ellipsometer (UVISEL HR460 from Horiba Scientific) at wavelengths ranging from 300 to 1500 nm with 1 nm interval. An incidence angle of 70° was used for all measurements. The raw signal measured by SE has the following form: $I(t) = I_0 + I_S \sin(\delta(t)) + I_C \cos(\delta(t))$, where $\delta(t)$ is a phase shift. In the experimental configuration, the values of I_0, I_C, and I_S are linked to the ellipsometric angles (Δ, Ψ) by the following relations: $I_0 = 1$, $I_S = \sin 2\Psi \sin \Delta$, $I_C = \sin 2\Psi \cos \Delta$. The ellipsometric angles Δ and Ψ are related to the complex reflection coefficients of polarized light: R_p and R_s for a polarization, respectively, parallel and perpendicular to the plane of incidence. For each sample, the measured spectra may be analyzed using an appropriate fitting model based on sample structure. All fitting steps were performed using the Delta-Psi Horiba software.

The scanning electron microscopy (SEM) investigations were carried out on a Hitachi S-4700 SEM FEG (field emission gun) operating with an acceleration voltage of 3 or 6 KV. A thin carbon film was sprayed onto the samples at least 2 h before setting them under the beam.

In Vitro Biological Evaluation: Cell Vitality Assay and Hemolysis Assay. These tests were performed following the International and European standards (ISO 10993-5/EN 30993-5)[29] with the human pulmonary microvascular endothelial cell line (HPMEC-ST1.6R).[30] HPMEC cells were cultured in Endothelial Cell Growth Medium MV (Promocell GmbH, Heidelberg, Germany) enriched with Endothelial Cell Growth SupplementMix (Promocell GmbH, Heidelberg, Germany),[31] streptomycin (0.1 g/L), and penicillin (100 IU/mL), at 37 °C in a CO_2 incubator (CB 150/APT line/Binder, LabExchange, Paris, France) with 5% CO_2/95% atmosphere and 100% relative humidity. The proliferation and vitality of cells were evaluated at 3 and 6 days as previously described.[32−34] Results were expressed as a percentage compared to the cell growth on tissue-culture polystyrene surface (TCPS).

A hemolysis test was performed according to ISO standards 10993-4.[35] A blood sample was collected in ethylene diamine tetraacetic acid (EDTA) BD Vacutainer tubes (Becton, Dickinson and Company) from a healthy donor (with their consent) and diluted in PBS to obtain blood substrate containing 10 $g{\cdot}L^{-1}$ of hemoglobin. One milliliter of the diluted blood was added to the multiwell culture plate containing the test samples ($n = 3$) or the controls (PBS diluted blood and 1% $NaCO_3$ diluted blood; $n = 3$). The culture plate was maintained in rotation (80 rpm) at 37 °C for 1 h. The suspension was then collected and centrifuged at 3000 rpm for 10 min and the free hemoglobin in the collected supernatants was measured by a spectrophotometer at 540 nm. The hemolytic index (HI), calculated as shown below, determined hemolytic activity of the sample in the diluted blood substrate.

$$\%\ \text{hemolysis} = (\text{OD}_{\text{test-sample}} - \text{OD}_{\text{negative-control}}) / (\text{OD}_{\text{positive-control}} - \text{OD}_{\text{negative-control}}) \quad \text{with}$$

$$(\text{OD} = \text{optical density})$$

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%.

Paclitaxel Quantification. PTX, an antiproliferative and immunosuppressive agent used in anticancer therapy, was chosen as the drug reference to test the functionalized platform. Its complexation with MeβCD has previously been studied elsewhere.[36] For each experiment, PTX was quantified by HPLC coupled to UV detection (HPLC-UV) (System Gold, Beckman-Coulter, Villepinte, France). Paclitaxel and docetaxel (internal standard) were separated with a reverse-phase column (C18, 5 μ, 110 A, 150 × 3 mm, Phenomenex Gemini) maintained at 40 °C. The mobile phase consisted of acetonitrile/NaH_2PO_4 0.02 M pH = 2 (40:60). The flow-rate was 0.5 mL/min, and the injection volume was 100 μL. Analytes were detected at 230 nm with a retention time of 19 and 24 min for docetaxel and paclitaxel, respectively.

Phase Solubility Diagram of PTX in Aqueous Solutions of MeβCD and polyCD. MeβCD and polyCD were dissolved in pure water at different concentrations (10−50 mg/mL) and 10 mg of PTX were added. After 12 h at RT under 80 rpm, the nondissolved PTX in solution was removed by filtration using a 0.45 μ PTFE filter membrane. The PTX concentration of the different solutions was determined by HPLC coupled with UV detection at 230 nm. PTX solubility diagrams were then plotted as the concentration of PTX in the aqueous phase with increasing MeβCD and polyCD concentrations.

Quantification of the PTX Complexed onto Coated CoCr Disks. Functionalized CoCr disks were immersed for 12 h in 5 mL of a 1 or 10 g/L PTX solution prepared in ethanol/water mixture (70/30, v/v). After drug adsorption, the disks were rinsed by simple immersion in water. Three milliliters of acetonitrile/water (40/60) mixture solution was added to desorb the drug for 12 h. The solution was filtered using a 0.45 μm PTFE filter membrane. The amount of PTX complexed onto disk samples was determined by HPLC.

Kinetic Release of PTX in Human Plasma. PTX adsorption by functionalized CoCr disks was carried out under the same conditions as mentioned above. After immersion in a concentrated PTX solution (10 g/L) overnight, disks were gently soaked in water in order to rinse off the excess. The disks were then immersed in a 5 mL human plasma solution (provided by Etablissement Français du Sang-Nord de France, Lille, France) under 80 rpm at 37 °C. The supernatant was completely removed at successive intervals ranging from 1 h to 7 days. Three disk samples for each group were collected, and 5 mL of human plasma was immediately added to the batch vessel to pursue the release. The disks were immersed in 3 mL of acetonitrile/water (40/60) mixture solution for 12 h to remove the PTX. This solution was filtered using a 0.45 μ PTFE filter membrane. The remaining amount of PTX on disk samples after 7 days of contact with blood plasma was determined by HPLC.

RESULTS AND DISCUSSION

CoCr Functionalization. To ensure strong adhesion of the cyclodextrin polymer onto the CoCr surface, a PDA polymer was used as a strong adhesive layer. This grafting strategy is based on the coating of a first PDA layer acting as a functionalized film integrating amino groups which promotes the adsorption and the chemical anchorage of a second layer composed of polyCD allowing the storage and the sustained release of drugs. PDA has frequently been used as a versatile adhesive platform for the immobilization of various biomolecules,[18] such as selenocystamine,[5] heparin,[37] SiRNA,[38] RGD (arginylglycylaspartic acid),[39] or VEGF (vascular endothelial growth factor),[40−42] peptides onto metallic surfaces. Indeed, this multifunctional coating contains reactive amino groups which can be engaged in coupling reaction[41] or PDA could be postfunctionalized via Shiff base or Michael addition reaction with thiol or amine containing molecules.[5,25,37,39,40] Recently, PDA was used for cardiovascular applications by Wang and coworkers who found that PDA coated onto 316 L stainless steel (SS) stents improved endothelial cell proliferation while smooth muscle cell proliferation was inhibited.[20,21] These results suggested that PDA could play a central role in the modification of vascular biomaterials. In addition, this coating showed good compliance to balloon expansion.[20,21] Bae et al. working on CoCr vascular stents have reported the development of a high thromboresistant coating using a dopamine-mediated heparin layer.[43] In accordance with these reports, we have used PDA to form thin surface-adherent films onto the

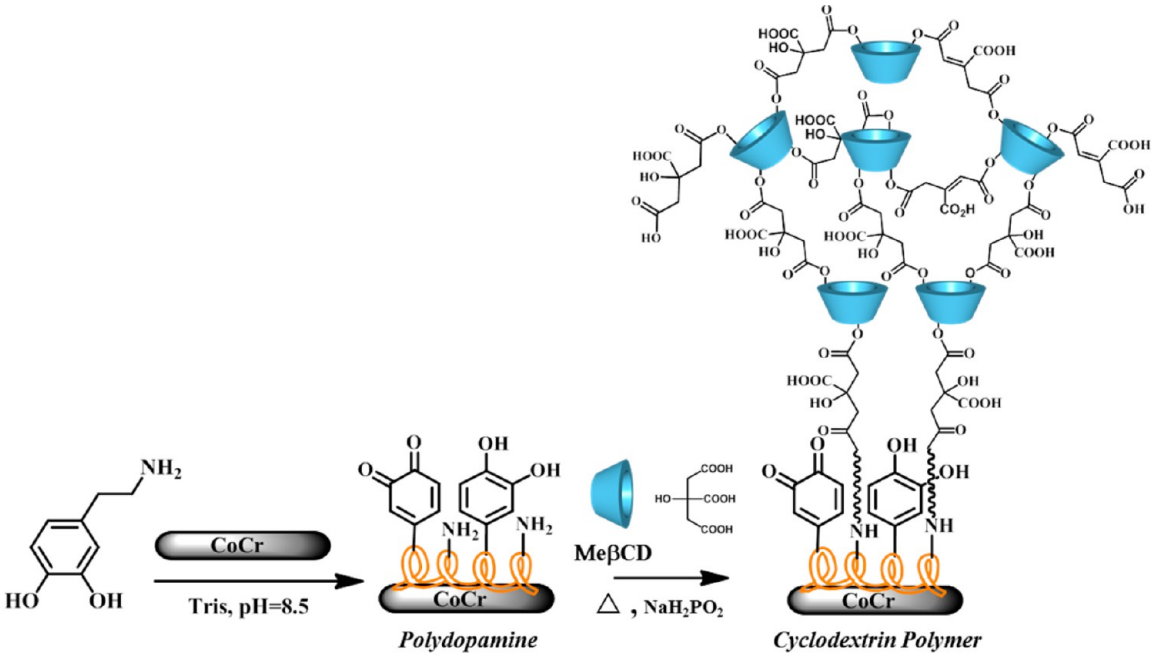

Figure 1. Representation of the strategy used for the functionalization of CoCr vascular device with cyclodextrin polymer.

CoCr surface and to promote the grafting of cyclodextrin polymer as described in Figure 1. Although the mechanism of PDA formation is not yet fully elucidated, this treatment results in the deposition of a PDA layer containing amino groups, essential for the further modification of the metallic surface.[38,44,45] Recently, significant efforts were devoted to understand the polydopamine structure[46−48] and the PDA deposition mechanism.[49] In the proposed PDA formation mechanism, the dopamine is oxidized into quinone under aqueous aerobic conditions and rearranged to form 5,6-dihydroxyindole (DHI).[50,51] This monomer and unreacted free-dopamine/quinone molecule react to form covalent aryl−aryl linkages[52] leading to the formation of a structure where amino groups remain exposed (Figure 1). An alternative structural model was proposed by Freeman et al. suggesting that PDA is a supramolecular aggregate of monomers held together through hydrogen bonding, π-stacking and charge transfer interactions.[46] Recently, further insights were brought by d'Ischia et al. who claim that polydopamine film is composed of three building blocks containing 5,6-dihydroxyindole units, uncyclized catecholamine/quinone moieties, and pyrrolecarboxylic acid fragments.[48] Finally, Lee et al. have found that a physical self-assembled trimer of dopamine$_2$/DHI is tightly entrapped within polydopamine film and may cause cytoxicity if released in the biologic environment.[47]

We hypothesized that the positively charged amino groups of PDA would be able to bind the negatively charged carboxylic groups of the additional layer of polyCD by ionic interactions. Furthermore, the strong adhesion of the polyCD layer to the PDA layer was also probably ensured by the formation of amide covalent bonds created between the carboxylic groups of the polyCD on the one hand, and the amino groups of PDA on the other hand.

Indeed, this coupling reaction occurs through the dehydration of the citric acid leading to anhydride intermediate which further reacts with the amino groups of the PDA coated layer and with the hydroxyls of the cyclodextrin unit. This reaction was promoted by the high temperature (140−160 °C) applied in the curing oven during the PCD coating procedure. In these conditions, water formed by the dehydration of the CTR was easily removed, with the reaction equilibrium being displaced toward the formation of a reticulated cyclodextrin polymer network (see Supporting Information Figure S1) onto the PDA functionalized CoCr surface.[22]

The PDA coating was achieved by immersion of the CoCr disks into a 5 mL solution of dopamine in Trizma buffer (10 mM) at pH adjusted to 8.5 as previously reported.[18] Dopamine at high pH formed a PDA−melanin-like layer that provided a surface with a large density of amino groups.[41] The amount of amino groups immobilized onto the CoCr surface before and after PDA functionalization was quantified by colorimetric titration by using orange acid II as dye (Figure 2).[53] UV−vis

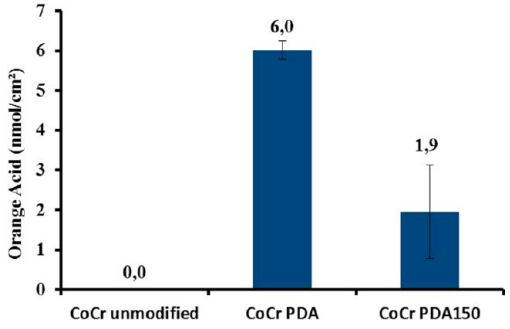

Figure 2. Orange Acid II colorimetric titrations of amino functions grafted onto CoCr surfaces with and without thermal treatment at 150 °C.

titrations indicate that the amount of grafted amino groups increased after impregnation in dopamine solution in accordance with the growth of PDA on the CoCr surface.[18] After 16 h of immersion, a density of 6 nmol/cm^2 of grafted amino functions was measured on the modified CoCr surface indicating a high PDA surface coverage. This value is a little higher compared to previously reported studies on titanium

and stainless steel surfaces and could be attributed to a thicker PDA layer obtained in the case of CoCr.[41]

Interestingly, it has been proved recently that an additional thermal treatment of the coated PDA film provides an improvement of the cytocompatibility of the endovascular device.[20] These results were attributed to the formation of quinone rich PDA allowing improvement of the biological properties. For this reason and keeping in mind that the objective is to enhance the long-term hemocompatibility of the stent device, CoCr PDA modified surfaces were subjected to a thermal treatment. However, we speculated that, during the thermal treatment, PDA incurred intramolecular cyclization and formed indole derivatives.[20] To ensure that amino groups remained after heating, OA colorimetric titrations were performed with the PDA coated CoCr surfaces. We can observe in Figure 2 that the amino content was divided by 3 after thermal treatment, indicating that a sufficient amount of amino functions remains available for the linkage with the cyclodextrin polymer after the heating step.

Cyclodextrin polymers are synthetic and biocompatible hydrophilic polymers obtained by polycondensation reactions between CD, a natural cyclic oligosaccharide, CTR as cross-linker, and sodium hypophosphite as catalyst. The polyCD synthesis process was first described by Martel et al.[22] and can be coated onto a wide range of biomaterials such as porous hydroxyapatite,[15] polyvinylidene difluoride (PVDF) membrane,[54,55] and in a more specific extent to vascular polyester textile grafts.[14,26,56,57] These applications showed that cyclodextrin polymers are efficient platforms designed for drug delivery and moreover, they are applicable to different biomaterials as long as the modification processes are specifically adapted. To date, the ability to graft polyCD to mirror-polished metallic surfaces remains unsuccessful, due to their absence of chemical reactivity.

According to previous published results,[17] the methylated β-Cyclodextrin (MeβCD) was selected for this study as it allows a more prolonged drug release kinetic and the best complexation constant with TBO and drugs compared to the other CD. MeβCD based polymers were generated "in situ" by impregnation of the CoCr PDA functionalized disks into a solution containing CTR, $Na_2H_2PO_2$ and MeβCD (ratio 10/3/10 in g/100 mL) for 10 min at RT. After drying at 90 °C for 30 min, the CoCr disks were subjected to a polycondensation reaction by heating. In this study, an optimization of several parameters such as treatment duration and temperature of curing was achieved. Most of the time, the optimization of the parameters was correlated with TBO quantification after the whole process of functionalization was achieved.

First, to improve the grafting of the PDA layer onto the metallic surface and to define the best parameters that promote a higher amount of grafted MeβCD-based polymer with the best kinetic release profile, the coated PDA layer was first subjected to several thermal treatments (Figure 3). For this purpose, CoCr samples treated with PDA at temperatures ranging from 150 to 180 °C were functionalized with the MeβCD and CTR, and the amount of resulting polyCD grafted onto the surface was quantified by colorimetric titrations using TBO as probe (Figure 3a). The formation of the polyCD layer onto the PDA coated CoCr disk samples was clearly demonstrated since a significant amount of adsorbed TBO was observed on these samples (660 nmol/cm^2) . It is important to note that a small amount of polyCD was measured in the case of unmodified CoCr surfaces (54 nmol/cm^2) but this layer appeared as nonhomogenous suggesting physical adsorption of the polyCD onto the metallic surface.

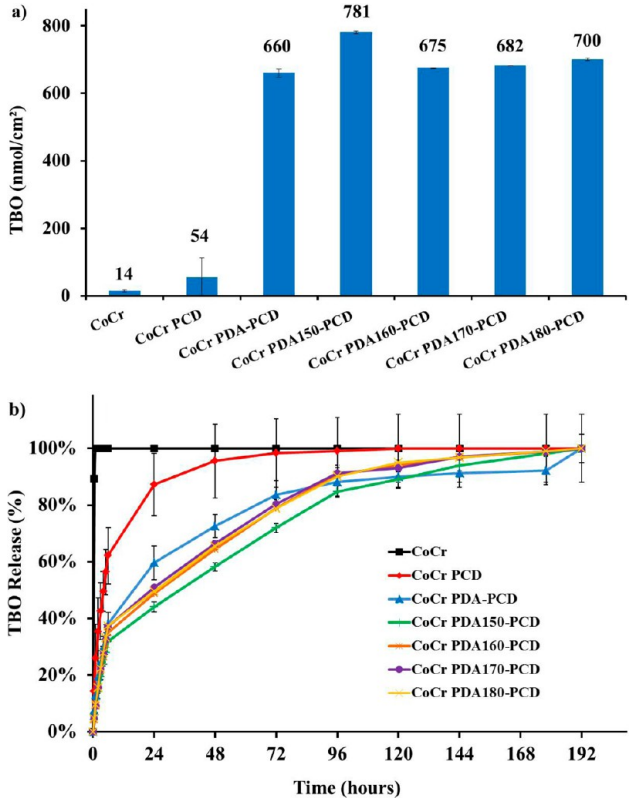

Figure 3. Influence of the PDA thermal treatment on the amount of polyCD grafting measured by TBO colorimetric titrations (a) and the kinetic release of TBO in PBS for several durations evaluated by UV–vis (b). For these experiments, according to previous studies, the polycondensation temperature of polyCD was fixed at 150 °C for 30 min.

This hypothesis was confirmed by immersion of the sample into a water and PBS solution, and after a few minutes of immersion, a detachment of the adsorbed layer was observed (data not shown) in both cases. With PDA coated disks, the amount of polyCD grafted onto the surface increased slightly after the thermal treatment with a maximum at T = 150 °C suggesting that this thermal treatment did not induce any significant enhancement of the grafting process.

Surprisingly, disks where thermal treatment was applied after PDA impregnation displayed a more homogeneous coating compared to those which had not undergone that curing after 6 days in PBS batch. As a matter of fact, we observed an improved adhesion of the cyclodextrin polymeric layer onto the CoCr substrate submitted to a PBS batch for 6 days when PDA was cured. As observed in Figure 4, no degradation of the coating was noticed on the disks that had undergone the thermal treatment. This result could be attributed to the rearrangement of the PDA layer during the thermal treatment leading to a more reticulated and to a more strongly attached polymer layer. Drug release kinetics evaluated in PBS solution and achieved by colorimetric titrations with TBO as a drug model further confirmed these observations (Figure 3b).

In Figure 3b, the release kinetics of the TBO dye were short (within 24–48 h) for the polyCD-coated disk samples not treated with a PDA layer. This trend could be attributed to the fast degradation of the polyCD adsorbed layer in PBS as

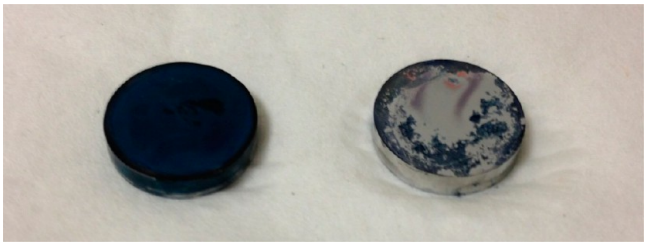

Figure 4. Images of CoCr plates functionalized with PDA and polyCD after 6 days in PBS batch: On the left side, a disk sample with PDA thermal treatment at 150 °C for 1 h; on the right side, a disk sample without PDA thermal treatment. (The polyCD coating was revealed by immersion in Toluidine Blue O solution.)

previously observed. The treatment of samples with the PDA layer induced a considerable increase in the release time from 24 to 100 h (×5). Furthermore, a significant enhancement of the drug-delivery period was observed after PDA thermal treatments and more particularly for samples treated at 150 °C, indicating the key role played both by the PDA adhesive layer and the thermal treatment. Thus, for the next experiments, CoCr samples were functionalized with PDA by immersion in a 2 mg/mL Dopamine solution in Trizma buffer at pH = 8.5 for 16 h and an additional heating treatment at 150 °C for 1 h was applied to ensure the grafting of the polyCD layer onto the surface.

Once it was demonstrated that the coating of the first PDA layer was required to create a strong attachment of the polyCD polymer onto the CoCr surface, the reaction conditions for the optimization of polyCD grafting were investigated in depth. For this purpose, several temperatures of polycondensation ranging from 140 to 160 °C (Figure 5a) and different reaction times (20, 30, 45, and 60 min) were tested (Figure 5b). Figure 5 shows the influence of these two parameters on the polyCD grafting. In these experiments, the PDA CoCr devices were preliminary dipped into a MeβCD/$Na_2H_2PO_2$/CTR mixture (ratio 10/3/10) before curing.

In Figure 5a, when the reaction time was fixed at 30 min, it was clearly observed that a temperature of at least 150 °C was required to achieve an efficient grafting of polyCD revealed by an optimal TBO sorption (554 nmol/cm^2) and a sustained drug release (4 days).

When the fixation temperature increases over 150 °C, the TBO sorption capacity of the disk samples decreased. In the same way, Figure 5b shows that the TBO titrations displayed an optimal curing duration of 30 min (543 nmol TBO/cm^2) when the reaction time was varied from 20 to 60 min. Further extension of the reaction time over 30 min resulted in a decrease of the TBO sorption on the modified CoCr plates. Indeed, as observed in Figure 5, the application of more drastic conditions (by increasing time and temperature of the curing) resulted in a decrease of TBO sorption on the modified CoCr surface. Those findings could be explained by an extension of the cross-linking reaction that would convert the residual free carboxylate groups into ester groups, preventing TBO sorption by ionic interactions on the CoCr surface and by the reduction of the MeβCD cavities with their subsequent inclusion possibilities.[58] Considering those results, we set the time and temperature of reaction respectively at 30 min and 150 °C.

Figure 5 also displays the release kinetics of TBO in PBS batch at 37 °C. When mild conditions were applied for the fixation of polyCD onto the CoCr-PDA modified surface (e.g.,

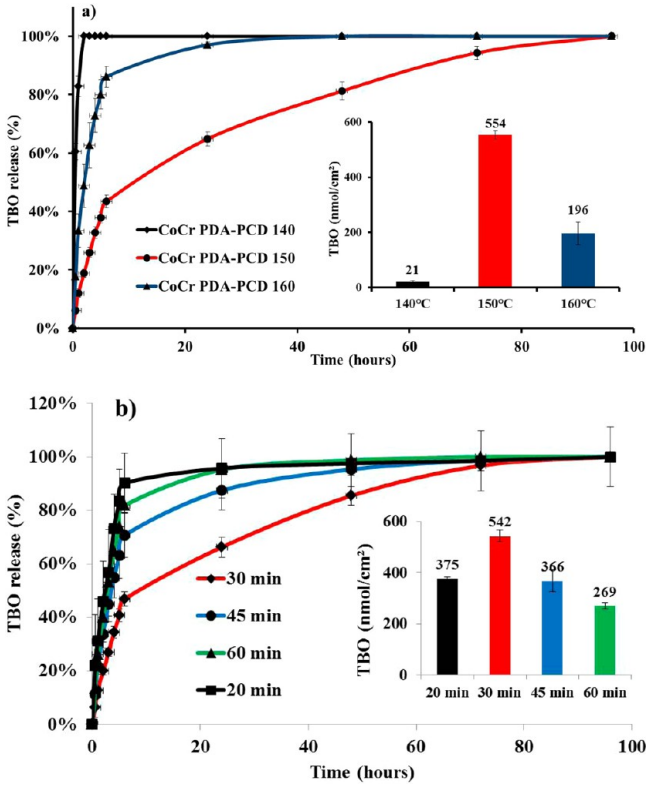

Figure 5. (a) Influence of the curing temperature (curing time = 30 min) and (b) influence of the curing time (curing temperature = 150 °C) on the polyCD grafting rate (inset histograms) determined by TBO quantification. Plots represent TBO release kinetic in PBS batches (pH 7.4, 37 °C, 80 rpm) in both experiment series.

140 °C, 30 min or 150 °C, 20 min), TBO was released more rapidly, with 100% of TBO eluted within 1−5 h. In comparison, when optimal conditions were applied as previously defined for the TBO sorption (e.g., 150 °C, 30 min), the elution lasted more than 100 h (i.e, more than 4 days). As soon as the parameters were modified with the increase of time and/or temperature of the curing (e.g., 160 °C, 30 min or 150 °C, 60 min), the release times of TBO was shortened.

Finally, it is worth mentioning that all kinetic curves presented two different phases: an immediate burst release followed by a slow release. The first phase (burst release) included the desorption of the TBO molecules due to a competition with the ions of the PBS medium that interact with the CoCr modified surface through ionic interactions. The second phase could be attributed to the slow release of the TBO molecules immobilized into the polyCD network through inclusion complex with the MeβCD cavities.[59]

Coating Morphologies. The morphologies of PDA and polyCD coating layers were investigated by scanning electron microscopy (SEM). SEM images confirmed the presence of the PDA coated layer on the CoCr surface (Figure 6, left) as a dense and homogeneous film was observed. However, some particles were observed on the PDA film and were assigned to the formation of PDA particles during the polymerization process.[60] The PDA film thickness was estimated from SEM images. For this purpose, a scratch was made with a Teflon tip on the CoCr coated layer and a film thickness of almost 70 nm was measured demonstrating the formation of a dense and thick PDA adhesive coated layer. The PDA coating was further characterized by ellipsometry, and a film thickness of 86 nm

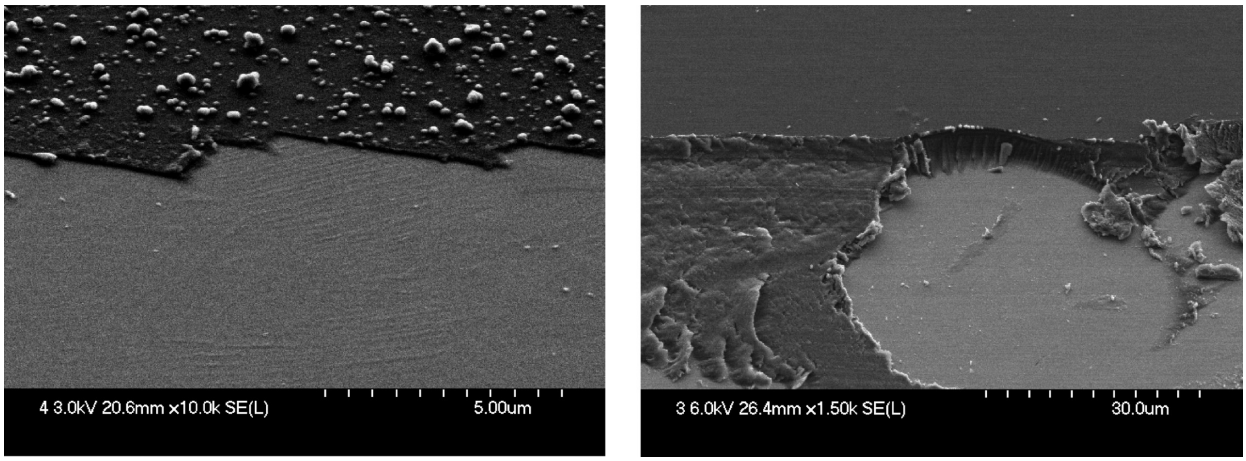

Figure 6. SEM Images of PDA coating layer on CoCr disks (left) and PDA/polyCD coating layer on CoCr disks (right).

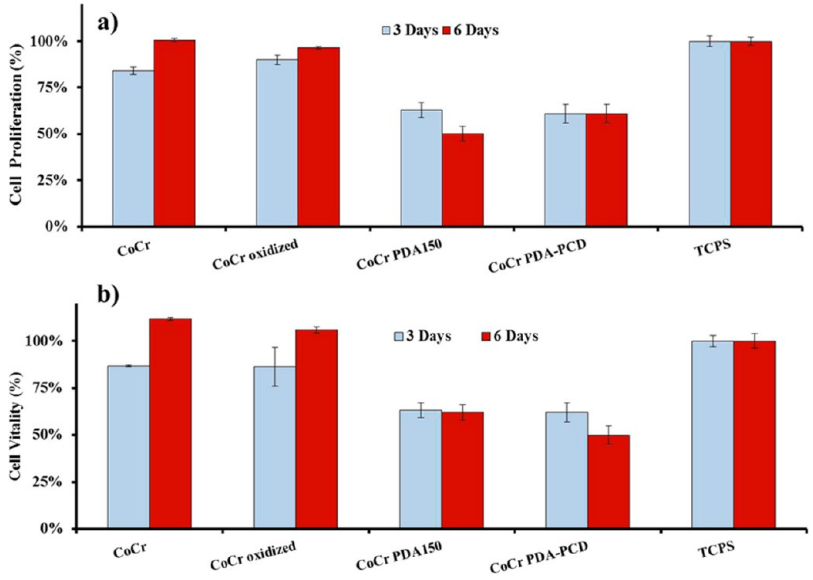

Figure 7. Assessment of proliferation by cell counting (a) and viability by Blue Alamar fluorescence (b) of HPMEC on functionalized CoCr disk samples tested at each step of the grafting process after 3 and 6 day culture (37 °C, 5% CO_2, 100% relative humidity), without renewal of the culture medium.

±0.5 was measured, which confirmed the SEM analyses. The thickness of this coated layer is higher than those observed on titanium or stainless steel surfaces[41] in the same conditions but is quite similar to the one observed on silicium.[18]

The SEM image of polyCD coating layer showed a dense and very homogeneous grafted polymer film on CoCr disks (Figure 6, right). A film thickness of 5.7 μm could be estimated from the SEM image. Unfortunately, the measurement of the polyCD film thickness was not possible by ellipsometry technique because light scattering phenomena were observed on these samples.

XPS Investigations. XPS investigations (see Supporting Information Figure S2) were performed on CoCr-PDA and CoCr-PDA-PCD functionalized materials. XPS survey spectra clearly showed the formation of the coated layer in both cases as carbon (at 285.0 eV), oxygen (at 533.1 eV), and nitrogen (at 400.2 eV) assigned to the polymers were observed, while no residual Co or Cr component was detected. In addition, the N1s core level spectra of the CoCr-PDA coated layer exhibits a single component at 400.2 eV which could be assigned to the presence of amino-derivative groups in the film.[21,41] Unfortunately, any evidence in the formation of the amide bond between PDA and PCD was demonstrated by XPS as no significant change in the shape of the N1s core level spectrum was observed after PCD grafting.

Biological Evaluation. In order to evaluate the cytocompatibility of the CoCr functionalized device, cell proliferation and vitality tests were performed on metallic surfaces at each step of the functionalization process (i.e., oxidation treatment, PDA and polyCD grafting) with HPMEC.[29]

As depicted in Figure 7, both vitality and proliferation of cells are favorable on CoCr and CoCr oxidized disks, which exhibit proliferation and vitality rates higher than 80% and 90% after 3 and 6 days, respectively. These results suggest that the oxidation step associated with an intensive rinsing sequence preserved the cytocompatibility of the CoCr device. The proliferation and vitality rates dropped, respectively, to 50% and 61% after PDA and polyCD grafting for 6 day cell culture. Those decreases could be attributed to the leach of uncoated dopamine fragments[61] or physical self-assembled trimer of dopamine$_2$/DHI[47] into the culture medium. In the same manner, the decrease of HPMEC proliferation observed for

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CoCr PDA150 samples within 3 and 6 days is probably due to the slow degradation of the polydopamine coating into the culture medium. Interestingly, HPMEC proliferation is very low if the plates do not undergo the oxidation treatment. As a consequence, these results were optimized by increasing the duration of the oxidation treatment which enhances interactions between hydroxyl groups generated on CoCr surface by oxidation and the catechol groups of the PDA layer.[24]

Biological evaluations performed on polyCD coated CoCr samples showed that the cyclodextrin polymer did not significantly affect HPMEC. A slight decrease of cell vitality was observed between 3 and 6 days, probably due to the degradation of the coated cyclodextrin polymer from the CoCr surface into the culture medium. Nevertheless, it can be postulated that this surface modification would not result in damage to human tissues.[62]

Hemolysis assays performed on the functionalized CoCr surfaces showed that functionalized CoCr disk samples did not induce hemolysis when placed in contact with human blood (HI = 0) (similar to that of PBS negative control).

Finally, the adsorption of plasma proteins onto the modified surface should also be considered in the case of implantable medical devices. This protein absorption is highly dependent on the surface wettability of the materials. The functionalization of the CoCr surfaces by the cyclodextrin based polymer increases the surface wettability of the biomaterials (contact angle decreases from 73° ± 4 for CoCr to 49° ± 4 for CoCr-PDA-PCD). This trend is attributed to the grafting of hydrophilic cyclodextrin units onto the CoCr surface and to the generation of hydrophilic carboxylic acid groups during the polycondensation reaction. The impact of this functionalization on the prosthesis biointegration was previously investigated on PCD grafted PET vascular devices.[62] Especially, in vivo studies have pointed out that the cyclodextrin based polymer grafted onto the vascular prosthesis is safe and did not show any significant differences in terms of tissue integration compared to the virgin prosthesis.

Degradation Study. Our group has recently established that cyclodextrin polymers coated on knitted polyester vascular prostheses were biodegradable.[62] It was specifically observed that polyCD was completely hydrolyzed in human plasma within 2 months in in vitro conditions while it takes 1−6 months when in vivo environments are considered. The biodegradation process of polyCD grafted onto the PDA modified CoCr surface was studied in culture medium. The amount of polyCD remaining on the CoCr surface as a function of immersion time was determined by TBO titrations (Figure 8). The polyCD content was significantly decreased by increasing the immersion time, suggesting that the polyCD coating was completely degraded after 96 h (4 days). In comparison with PET vascular prosthesis,[14] the degradation of the polyCD layer grafted onto CoCr surface was probably more due to its bulk detachment rather than hydrolysis process. This observation could be attributed to the presence of strong ionic forces induced by the culture medium. We can hypothesize that the TBO release profile observed in Figure 5 was controlled by polyCD layer removal and not by biodegradation of the coating layer as observed previously.[17] These results further corroborate the hypothesis of a detachment of the polyCD leading to a partial cytotoxicity of polyCD coated surfaces (Figure 7).

Paclitaxel. PTX, a highly hydrophobic anticancer agent, is currently considered as the reference drug to limit restenosis.[63] Cyclodextrins, due to the lipophilic nature of their cavity, are

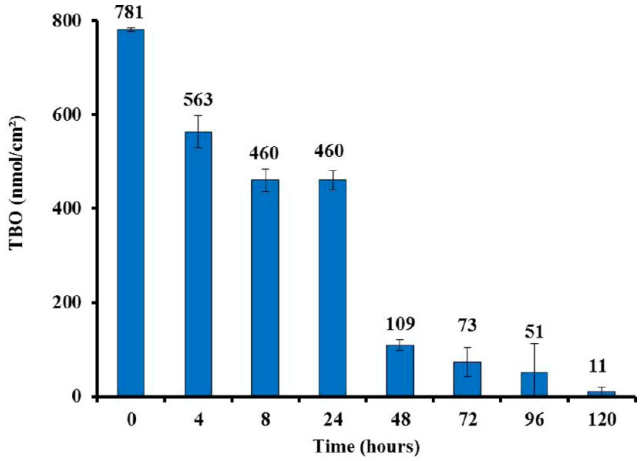

Figure 8. Degradation studies performed by UV−vis titrations on PDA150 modified CoCr surfaces functionalized by polyCD in culture medium at 37 °C subjected to shaking (80 rpm).

commonly used to improve the aqueous solubility, the stability and the bioavailability of hydrophobic drugs.[10]

Even though the complexation between PTX and MeβCD has already been described elsewhere,[64−66] we have investigated the improvement of PTX solubility in water in the presence of MeβCD and polyCD. Therefore, Figure 9 shows the drug

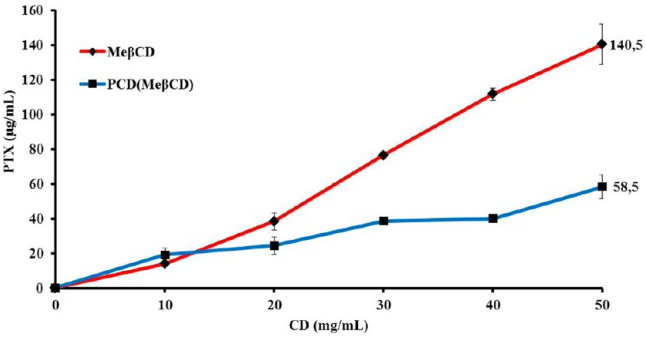

Figure 9. Phase solubility diagram of PTX in MeβCD and polyCD aqueous solutions.

solubility diagrams of PTX both in MeβCD and polyCD aqueous solutions. As predicted, the poor solubility of PTX in water (0.7 mg/L) was significantly increased in the presence of MeβCD and polyCD. For instance, the PTX solubility was increased by 180 (140 mg/L) and 90 (60 mg/L) in a 50 g/L aqueous solution of MeβCD and polyCD, respectively. At equivalent concentrations, MeβCD could solubilize two times more PTX compared to its polymerized form (i.e., polyCD), which is composed of a 50/50 ratio in weight of MeβCD and CTR moieties.[26] Therefore, the latter contains two times less cyclodextrin cavities available to complex with PTX.

In conclusion, PTX solubility diagrams confirmed the ability of MeβCD to complex with PTX in an aqueous medium. Besides, that property was not affected by the MeβCD cross-linking with CTR. As a consequence, one can expect that PTX will be adsorbed on the modified CoCr surface through its interaction with the polyCD layer.

We investigated the ability of the polyCD coated CoCr device to release the paclitaxel drug. For this purpose, disk samples were loaded by immersion in a PTX ethanolic solution for 12 h and rinsed in pure water afterward to precipitate the

nonadsorbed PTX. Interestingly, the cyclodextrin polymer grafted onto the CoCr surface allowed a drug sorption in the range of 0.6−9 $\mu g/mm^2$ by adjusting the concentration of the impregnation solution in the range of 1−10 g/L (data not shown).

The CoCr functionalized samples loaded with PTX (~9 $\mu g/mm^2$) were placed in human plasma and the amount of PTX released in solution as a function of the release time was assessed by HPLC measurements (Figure 10). As positive

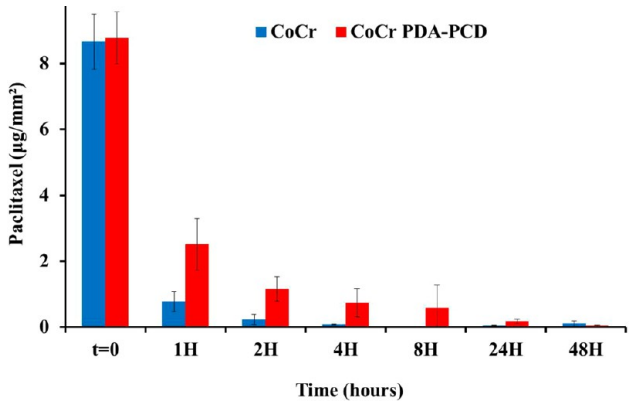

Figure 10. Evaluation of the remaining amount of PTX on the CoCr and PDA-polyCD coated CoCr disks (⌀14.5 mm) after different durations of stay in human plasma at 37 °C subjected to shaking (80 rpm).

control, the same amount of PTX was intentionally deposited onto unmodified CoCr disks. It is important to acknowledge that PTX is a highly lipophilic drug which can be simply adsorbed onto the metallic surface by dip coating or by spraying. This property is the key point of the concept of the polymer-free-eluting vascular stent currently available in clinical practice with a drug sorption limited to 3 $\mu g/mL$.[67]

The main relevant aspect of this drug-eluting system was to extend the release of the PTX drug from the polymeric platform compared to the uncoated device.

As observed in Figure 10, HPLC measurements showed a quick release of 91% of the total PTX loaded from the CoCr unmodified samples within the first hour. This value was limited to 71% with PDA-PCD CoCr functionalized samples. Nevertheless, a burst effect was observed with the PCD coated layer and was attributed to the release of some PTX molecules which are probably entangled within the cross-linked polymer networks and immediately released. In addition, Figure 10 shows that the whole release of PTX from the control disk sample was achieved within the first hour following its immersion in plasma, while higher amount of PTX could be still detectable in plasma within 24 h with the polyCD modified CoCr surface. This result highlighted the reservoir role played by the cyclodextrin polymer grafted onto the CoCr surface in the release of PTX.

As a matter of fact, the PTX sorption onto the bare CoCr surface could be attributed to the high lipophilicity of the PTX promoting its spontaneous adherence onto hydrophobic surfaces and leading to the fast desorption of PTX in plasma. On the contrary, polyCD coated CoCr surfaces interacted with PTX through the polymer network and cyclodextrin inclusion complexes leading to the PTX sustained release in a superior extent than the control surface. However, the release kinetic looks not significantly lower than the unmodified materials. This trend could be explained by the weak host−guest interactions[36] observed between the MeβCD and PTX (i.e., $K_a \sim 400\ M^{-1}$) or/and by the substitution of the PTX from the CD-cavities by the plasma constituents including lipid and cholesterol.[58,68]

We thus concluded that the polyCD CoCr functionalized stent will hold more PTX over time compared to bare material and will prevent intrastent restenosis. These hypothesis need to be confirmed by using the in vivo model.[69]

Finally, we attempted to functionalize a CoCr vascular stent (Multi-linkVision, AbbottVascular, Inc., Santa Clara, CA) by using this coating strategy. The grafting of the two successive layers (PDA and polyCD) was assessed by SEM. On the SEM images (Figure 11, left), we can observe the appearance of a rough coating layer indicating the formation of a thin film of PDA on the stent (Figure 11a and inset). The further functionalization of the PDA-coated stent by the cyclodextrin polymer leads to the formation of a dense and homogeneous polymer layer on the stent (Figure 11b). Interestingly, the polymer layer completely covered the stent and inspection of the coated device by SEM did not reveal the presence of cracks or defects. These functionalized CoCr vascular stents will soon be implanted in the aorta of a rat model developed in our research unit department.[69] Our aim is to confirm the innocuousness and the safety of the CoCr stent functionalized by the cyclodextrin coated layer and to assess its potential effect on intrastent restenosis.[69]

CONCLUSION

In conclusion, the technological barrier resolved in this study concerned the grafting of a cyclodextrin based polymer onto

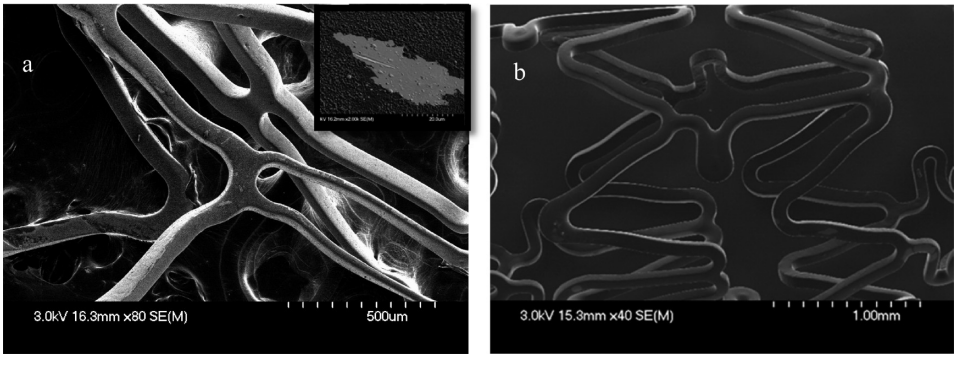

Figure 11. SEM Image of PDA coating layer (a) and PDA/polyCD coating layer onto CoCr vascular stents (b).

mirror polished CoCr surfaces. The proposed solution was based on the pretreatment of CoCr substrates with polydopamine, a bioinspired polymer acting as strong adhesive and chemically reactive layer promoting the cyclodextrin polymer anchorage. The experimental conditions for this latter step were optimized, and it was shown that the curing at 150 °C for 30 min led to the optimal loading of polyCD onto the CoCr surface with the sustained delivery of TBO which was chosen for the cyclodextrin polymer titration and as a drug model.

In vitro evaluations showed that this coated layer is hemo- and cyto-compatible. Furthermore, the ability of this cyclodextrin polymer coating to hold more PTX over time compared to the uncoated device was demonstrated. These results evidenced the promising potential of such systems to act as drug-delivery systems. Moreover, this grafting strategy was successfully extended to a commercial CoCr vascular stent. Further in vivo tests on rats are under investigation to evaluate the potential of such coated devices to elute several hydrophobic drugs promoting arterial wall healing.

■ ASSOCIATED CONTENT

Supporting Information

(1) Description of the cyclodextrin based polymer formation mechanism (Figure S1); (2) XPS spectra relative to CoCr-PDA and CoCr-PDA-PCD grafting layers (Figure S2). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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