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Bioinspired Titanium Drug Eluting Platforms Based on a Poly-β-cyclodextrin–Chitosan Layer-by-Layer Self-Assembly Targeting Infections

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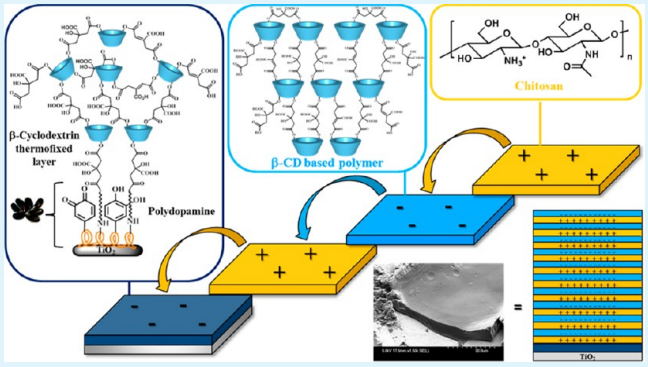
Supporting Information

ABSTRACT: In the field of implantable titanium-based biomaterials, infections and inflammations are the most common forms of postoperative complications. The controlled local delivery of therapeutics from implants through polyelectrolyte multilayers (PEMs) has recently emerged as a versatile technique that has shown great promise in the transformation of a classical medical implant into a drug delivery system. Herein, we report the design and the elaboration of new biodegradable multidrug-eluting titanium platforms based on a polyelectrolyte multilayer bioactive coating that target infections. These systems were built up in mild conditions according to the layer-by-layer (L-b-L) assembly and incorporate two biocompatible polysaccharides held together through electrostatic interactions. A synthetic, negatively charged β-cyclodextrin-based polymer (PCD), well-known for forming stable and reversible complexes with hydrophobic therapeutic agents, was exploited as a multidrug reservoir, and chitosan (CHT), a naturally occurring, positively charged polyelectrolyte, was used as a barrier for controlling the drug delivery rate. These polyelectrolyte multilayer films were strongly attached to the titanium surface through a bioinspired polydopamine (PDA) film acting as an adhesive first layer and promoting the robust anchorage of PEMs onto the biomaterials. Prior to the multilayer film deposition, the interactions between both oppositely charged polyelectrolytes, as well the multilayer growth, were monitored by employing surface plasmon resonance (SPR). Several PEMs integrating 5, 10, and 15 bilayers were engineered using the dip coating strategy, and the polyelectrolyte surface densities were estimated by colorimetric titrations and gravimetric analyses. The morphologies of these multilayer systems, as well as their naturally occurring degradation in a physiological medium, were investigated by scanning electron microscopy (SEM), and their thicknesses were measured by means of profilometry and ellipsometry studies. Finally, the ability of the coated titanium multilayer devices to act as a drug-eluting system and to treat infections was validated with gentamicin, a relevant water-soluble antibiotic commonly used in medicine due to its broad bactericidal spectrum.



KEYWORDS: *titanium, polyelectrolyte multilayer, cyclodextrin-based polymer, polydopamine, chitosan, drug delivery system, antibacterial coating*

INTRODUCTION

Titanium and its alloys, owing to their exceptional mechanical, chemical, and biological properties, are widely used in biomedical applications for the conception of prosthetic implants including dental, vascular, and orthopedic implants.[1] However, early failures in implants were usually observed due to surgical- and implant-related complications such as inflammations and infections, thereby involving long-term post-operatory problems and mortality in the worst cases. The treatment of those infections requires four to six weeks of

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antibiotic therapy and two additional surgical operations, thus increasing the economic and health-related costs.

In this context, many efforts were devoted to altering surface properties with the aim of improving the titanium-based implants' performance and their integration in bone and tissue. Several approaches then emerged for surface engineering. including hydroxyapatite coating,[2] plasma-induced grafting,[3] sol−gel,[4] electrodeposition,[5] and the covalent attachment of target molecules[6−9] or polymers.[10−12] Among these aforementioned techniques, Layer-by-Layer (L-b-L) self-assembly, based on the consecutive absorption of polyanion and polycation electrolytes, was found to be one of the simplest and most popular approaches for designing bioactive multilayer coatings and has recently emerged as a promising technique for engineering drug delivery systems under mild conditions.[13,14]

Over the past two decades, a plethora of structurally diverse synthetic and natural polymers were utilized to create multilayered films. Chitosan (CHT), a natural and biocompatible polymer, was extensively used for L-b-L preparation because it shows intrinsic antimicrobial and hemocompatible properties[15] and could be easily incorporated into L-b-L systems via electrostatic interactions. In addition, suitable polymeric materials could be combined with CHT to provide new functionalities such as a drug reservoir, for instance.[16−18]

In this context, the controlled local delivery of antibiotics appears as an attractive therapeutic approach to address perioperative infections within few days after the prosthesis implantation. For this purpose, polyelectrolyte multilayers (PEMs) are promising tools for the elaboration of drug delivery systems because they allow the confinement of bioactive molecules while preserving their bioactivity and delivering them locally.[19] Furthermore, PEMs offer the advantage of delivering larger amounts of drugs compared to that delivered using the Langmuir−Blodgett and SAM deposition techniques.[20] Unfortunately, in some cases, the direct incorporation of drugs into PEMs is limited and may lead to the disruption of the multilayer structure, thereby limiting their applications in drug delivery systems. Furthermore, an additional critical point of these multilayer assemblies' elaboration concerns the robustness of their anchorage on TiO_2 surfaces in physiological and dynamic conditions.[21]

Cyclodextrins, due to the hydrophobic character of their cavities,[22−25] are well-known for forming reversible inclusion complexes with many drugs including antibiotics and are widely used for their encapsulation properties as drug carriers.[26−28] Jessel et al.[29] pioneered the incorporation of the cyclodextrin (CD) unit as molecular chaperone into a L-b-L system to elaborate anti-inflammatory films. More recently, intensive works have been developed by Hammond et al. in the design of multidrug release coatings based on L-b-L assemblies.[30] More importantly, these authors have shown that a negatively charged CD-based polymer could be used to create drug release platforms[31,32] capable of targeting infection and inflammation.

A few years ago, Martel et al. developed the synthesis of a water-soluble, biocompatible, hemocompatible, and biodegradable CD-based polymer obtained by a polycondensation reaction between β-CD, citric acid (CTR) (as cross-linker), and sodium hypophosphite (as catalyst) and acting as drug reservoir for the sustained release of drugs.[33] This negatively charged polymer was grafted by a curing process onto a wide range of biomaterials including porous hydroxyapatite,[34,35] visceral meshes,[36] polyvinylidene difluoride membrane,[37,38] and, in a more specific extent, to polyethylene terephthalate (PET) vascular textile graft.[39−42] In such materials, the CD polymer attached to the support via physical interactions acts as a reservoir for the sustained release of one or more bioactive molecules. Recently, Martel et al. have proposed an evolution of such CD-based systems by taking advantage of the anionic character of the coating layer on textile fibers to build up a self-assembled multilayer system based on chitosan, on the one hand, and also a water-soluble β-cyclodextrin polymer cross-linked with citric acid, on the other hand.[43,44] In parallel, we have also developed an original approach involving the grafting of a CD-based polymer (PCD) onto cobalt−chromium vascular implants.[45] For this purpose, polydopamine (PDA), a strongly adhesive hemocompatible and biocompatible biopolymer,[46,47] was applied as a first coating layer onto the surface of the metallic CoCr device to promote the strong anchorage of the CD−CTR-based polymer generated in situ through a polycondensation reaction. Interestingly, Yang et al.,[21] inspired by the Messersmith group's pioneering works,[48] have recently proposed a biomimetic approach based on the grafting of a dopamine-modified hyaluronic acid to improve the binding strength between titanium alloys and a polyelectrolyte system composed of hyaluronic acid−CHT multilayers, thereby further confirming the efficiency of dopamine derivatives in the strong attachment of the multilayer systems onto the titanium-based biomaterials.

In this paper, we have combined the unique biological properties of CHT, the adhesive properties of PDA, and the reservoir features toward drugs of CD-based polymers to elaborate bioinspired and biodegradable multidrug eluting platforms. In particular, polyelectrolyte multilayers, which alternate the biocompatible CHT and PCD polymers, were grafted onto titanium surfaces and loaded with gentamicin to address perioperative infections.

EXPERIMENTAL SECTION

Materials. All reagents were purchased from Sigma-Aldrich (St. Louis, MO) and were used as received except for the native β-CD and gentamicin, which were provided by Roquette (Lestrem, France) and PANPHARMA (80 mg/2 mL), respectively.

The deacetylation degree (85%) of chitosan (low molecular weight grade, 59 200 < Mw < 65 000 g mol^{-1} determined by viscosimetry) was determined by UV−vis spectrophotometry using *N*-acetylglucosamine as a reference in hydrochloric acid.[49] This value corresponds to 5 mmol of cationic ammonium functions per g of chitosan. The CD-based polymer (PCD, Mn = 45 000 ± 5000 g mol^{-1}, 4 mmol anionic functions per gram of PCD) was obtained by a polycondensation reaction between citric acid and β−cyclodextrin according to a previously reported procedure.[33,50] Ultrapure water (18.2 MΩ, Millipore Milli-Q system, Merck KGaA, Germany) was used for all of the experiments.

Analytical Techniques. *Spectroscopic Ellipsometry.* Spectroscopic ellipsometry (SE) measurements were taken at room temperature using a phase-modulated ellipsometer (UVISEL HR460 from Horiba Scientific) at wavelengths ranging from 300 to 1500 nm with a 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 (Rp and Rs for a polarization that is, 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 the sample

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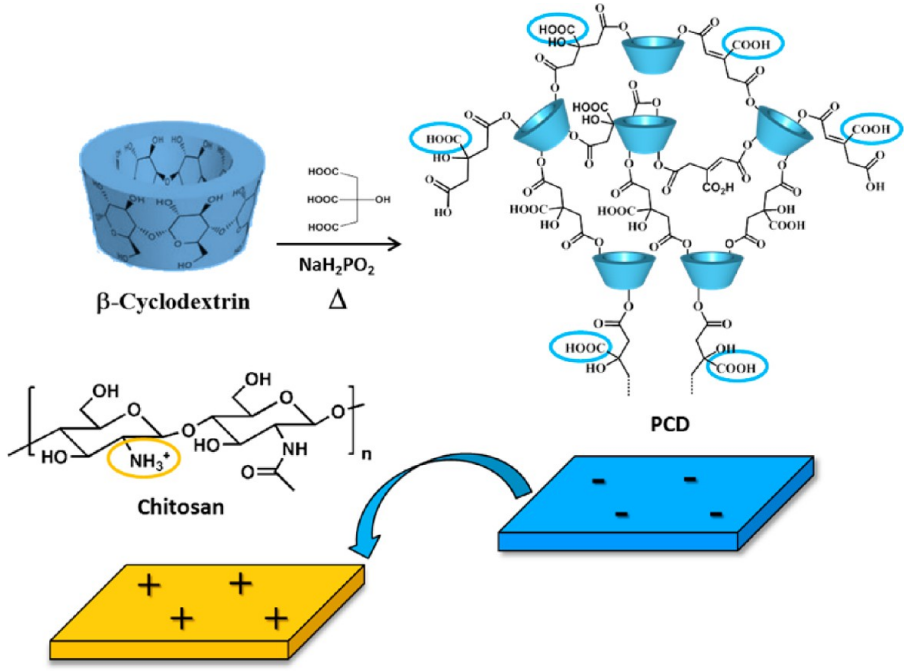
Scheme 1. Description of the Polyelectrolytes Involved in the Design of the Multilayer System



structure. All fitting steps were performed using the DeltaPsi Horiba software.

Mechanical Profilometry. The thickness of the coatings was evaluated by surface profilometry using an Alpha-Step IQ surface profiler (3 measures per samples). The Alpha-Step IQ is a mechanical, stylus-based step profiler that can measure step heights from several nanometers up to 2 mm.

Scanning Electron Microscopy. Investigations using scanning electron microscopy (SEM) 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.

Surface Plasmon Resonance. Measurements using surface plasmon resonance (SPR) were taken using a monochannel AutoLab Springle instrument (Eco Chemie, Netherlands). Briefly, polarized laser light (λ = 670 nm) is directed to the bottom side of the titanium disk (diameter of 2.54 cm) sensor via a hemispheric lens placed on a prism (BK7 having a refractive index of n = 1.52), and the reflected light is detected using a photodiode. An autosampler (Eco Chemie, The Netherlands) is used to inject or remove the tested solutions. All SPR measurements were done in nonflowing liquid conditions, i.e., with the circulating pump paused and at 20 °C. The noise level of the SPR angle is ∼1 millidegree. After each addition, the cell was thoroughly washed with the buffer solution. All measurements were conducted at 20 °C. The substrates were allowed to equilibrate until a steady baseline was observed. The anionic carboxylic acid layer was prepared by soaking the TiO_2 SPR wafer into a 2 mg/mL dopamine Tris buffer solution for 1 h and subsequent immersion in a Tris buffer solution (pH 8.5) of 3-mercaptopropionic acid (15 mg/mL) overnight.[51] After functionalization, the surface was washed with water and dried with nitrogen flow. CHT and PCD (0.002% w/w) aqueous solutions (NaCl 0.15 M) adjusted to pH 4.8 were used for the SPR studies.

UV–Vis. A Varian Cary 50 Scan UV–vis spectrophotometer was used for UV–vis studies.

Gravimetry. The weight gain of the functionalized titanium plates was measured with an ultra-microbalance from Kern with an accuracy of 0.01 mg.

TBO Titration. The quantification of grafted PCD onto the metallic disk surfaces was evaluated with Toluidine Blue Ortho (TBO) by UV–visible titration.[52] On the one hand, the carboxylic acid functions of the PCD interact with the cationic site of the TBO molecule through an ion exchange mechanism. On the other hand, TBO is also entrapped in the β-CD cavities according to its polycyclic and aromatic structure.[53] TBO is an aromatic blue dye whose quantification is determined by UV–visible spectrophotometry at 641 nm after standardization.[53,54] In this study, TBO titrations were used to quantify the amount of cyclodextrin polymer grafted onto the TiO_2 surfaces and as a drug model to evaluate the sustained kinetic release properties of the modified titanium plates. 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 room temperature. After 4 h of impregnation, the excess of TBO that was loaded but uncomplexed 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% v/v; peak absorbance, λ = 641 nm). The TBO release kinetic as well the coating degradation studies were assessed in 10 mL of phosphate-buffered saline solution at pH 7.4 and were analyzed by UV–vis spectrometry at λ = 288 nm.

Acid Orange Titration. Acid orange (AO) colorimetric titrations were used to quantify the amount of amino groups (from chitosan) grafted onto the modified titanium surfaces.[54,55] A 2.5×10^{-2} M acid orange solution was prepared from distilled water and adjusted to pH 3 with a 0.5 M HCl solution. Each sample was dipped into 10 mL of this solution overnight at 25°C under stirring (60 rpm) to adsorb AO. Samples were then washed twice with 20 mL of acidic water (pH 3, adjusted with 0.5 M HCl) for 5 min. Desorption occurred by immersing the samples into 10 mL of a solution at pH 12 (adjusted with 0.5 M NaOH) for 24 h at 25 °C under stirring. Finally, the pH of each solution was adjusted to 3 with 1 mL of HCl 0.1 M, and absorbance was measured at 485 nm.

Preparation of TiO_2 Substrates. Titanium plates (diameter of 1.49 cm) were first treated with an acidic oxidizing solution of concentrated sulfuric acid and hydrogen peroxide H_2SO_4/H_2O_2 (1:1 v/v) for 2 min to generate the corresponding hydroxylated titanium dioxide surface. Titanium plates were thoroughly rinsed with water, acetone, and ethanol, and dried under nitrogen flow before functionalization.

Preparation of TiO_2–PDA Surfaces. The pretreated titanium surfaces were then soaked in a dopamine solution (2 mg/mL) in Tris buffer (10 mM) adjusted to pH 8.5.[46] The reaction was performed at room temperature under 400 rpm for 24 h. The PDA-treated titanium

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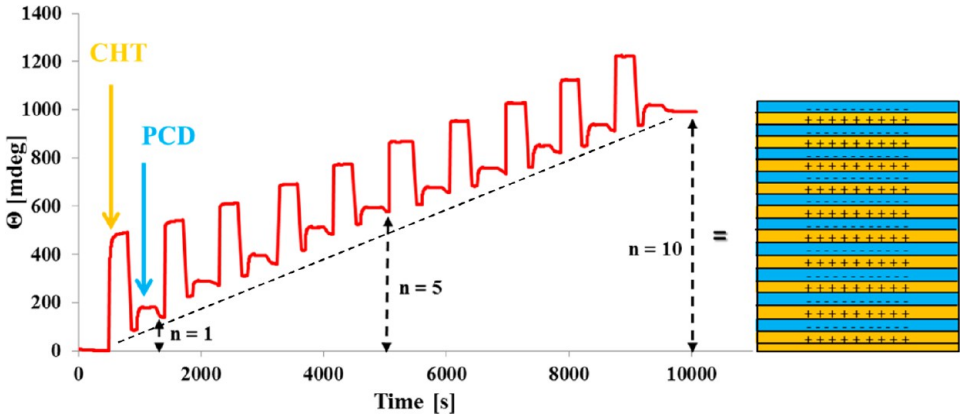

Figure 1. SPR sensorgram recorded on a COOH-functionalized TiO_2 surface during the subsequent injection of CHT and PCD polyelectrolytes (0.002% w/w in 0.15 M NaCl at pH 4.8).

plates were rinsed with deionized water, dried with air, and placed in a clean ventilated oven for a thermal treatment at 160 °C for 1 h.[45]

Preparation of a Thermofixed Cyclodextrin-Based Polymer Layer (TiO_2–PCD). Disk samples previously functionalized with PDA were immerged in a CTR/NaH_2PO_2/β-CD solution with a 10:3:10 ratio (g/100 mL of water) for 10 min at 37 °C (80 rpm) and were dried in a ventilated oven for 1 h at 90 °C. Sodium hypophosphite (NaH_2PO_2) was used as a catalyst, and CTR was used as a cross-linker. Thereafter, the polycondensation of β-CD and CTR was performed by curing at 140 °C for 30 min. Finally, the functionalized titanium surfaces were rinsed twice in ultrapure water. This process resulted in the fixation of a PCD rich in carboxylic acid groups on the PDA layer denoted in this study as TiO_2–PCD.

Preparation of Multilayer Assemblies. Before functionalization, PCD-functionalized titanium surfaces were rinsed three times with a phosphate buffer solution (0.25 M KH_2PO_4) adjusted to pH 4.8 to obtain the precursor charged layer. CHT–PCD multilayers were then prepared by subsequent immersion in CHT (0.5% w/w in 1% (v/v) acetic acid/water solution) and PCD (0.5% w/w in water) solutions at room temperature and pH 4.8 for 30 min under stirring (60 rpm).[43] Between each layer deposition, the samples were washed with the phosphate buffer solution for 2 min and dried under nitrogen flow. The process was repeated until the desired number of bilayers (n) was obtained. After the PEMs deposition, the surface was rinsed three times with ultrapure water and dried for 5 min at 90 °C.

Antibacterial Activity of the Gentamicin-Loaded Multilayer System. Gentamicin was loaded on each sample by soaking the titanium disk samples in a gentamicin pharmaceutic solution (40 mg mL^{-1}, Panpharma, France) for 4 h. The gentamicin-loaded disk samples were then soaked in PBS (2 mL) under shaking at 80 rpm and 37 °C with PBS renewal at regular intervals. The supernatants containing released gentamicin were placed on *Staphylococcus aureus* (S. aureus, CIP 224) preinoculated agar plates (Mueller-Hinton, BioRad, France) according to Kirby–Bauer disk diffusion susceptibility test.[56] After 24 h of incubation at 37 °C, the radius of the inhibition zone was measured and plotted as a function of the release time in PBS.

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

RESULTS AND DISCUSSION

In the design of the multilayer system, an anionic and biocompatible cyclodextrin-based polymer[57] was used for drug storage due to the ability of cyclodextrins to form inclusion complexes with drugs. Moreover, CHT, a natural polysaccharide produced by the deacetylation of chitin, was used as the polycation and acted as drug barrier to allow the sustained release of drugs (Scheme 1). These polyelectrolytes are oppositely charged polysaccharides that facilitate the growth of the multilayer system by self-assembly according to the L-b-L deposition principle.

Surface Plasmon Resonance. The ability of these two polyelectrolytes to self-assemble into multilayers through electrostatic interactions was first evidenced by using SPR. Indeed, SPR is a noninvasive optical method commonly used for biological applications that are sensitive to changes in refractive index and thickness in the close vicinity of the metal layer.[58] Here, we exploited SPR to study the binding events between polymers and the surface and to assess the formation of the multilayer assemblies by employing CHT and PCD as polyelectrolytes.

For this purpose, an aqueous CHT solution (0.002% w/w, NaCl 0.15 M, pH 4.8) was first injected onto a TiO_2 SPR wafer functionalized with an anionic carboxylic acid layer (Figure 1). Then, after flushing the SPR cell with the NaCl buffer solution (NaCl 0.15 M, pH 4.8), a shift of the resonance angle (Θ) was clearly observed, indicating the immobilization of a CHT layer onto the metallic anionic support. It is noteworthy that no change of the SPR angle was observed after two subsequent injections of CHT onto the first cationic layer (see Figure S1 in the Supporting Information), demonstrating that the adsorption of CHT onto the anionic layer occurs due to the complementary cationic character of the CHT polyelectrolyte.

Interestingly, upon the injection of an aqueous solution of the anionic CD-based polymer (0.002% w/w, pH 4.8, Mn = 45 000 g mol^{-1}) to the precoated layer and after the rinsing step with NaCl buffer solution, an increase of the reflectivity (Θ) is evidenced according to the immobilization of the PCD onto the CHT layer and the formation of a polyelectrolyte bilayer onto the titanium wafer (n = 1). As depicted in Figure S1 in the Supporting Information, no change of reflectivity was observed after the subsequent injection of PCD onto the first bilayer, which indicates that the growth of the multilayer system is mainly driven by electrostatic interactions. Finally, after subsequent injections of oppositely charged polyelectrolytes, the formation of multilayer assemblies integrating 5 and 10 bilayers was clearly evidenced, thereby demonstrating the ability of PCD and CHT to form PEMs in mild conditions.

Coating of the Cyclodextrin-Based Thermofixed Layer (TiO_2–PCD). Once the formation of the multilayer system on the titanium-based SPR wafer was achieved, the next challenge was to apply this strategy to the metallic device. In the past few years, dopamine has emerged as an important and versatile

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Scheme 2. Coating of an Anionic β-CD-Based Thermofixed Layer onto a Titanium Surface via PDA (COOH Surface Quantifications with TBO Shown in Insert)

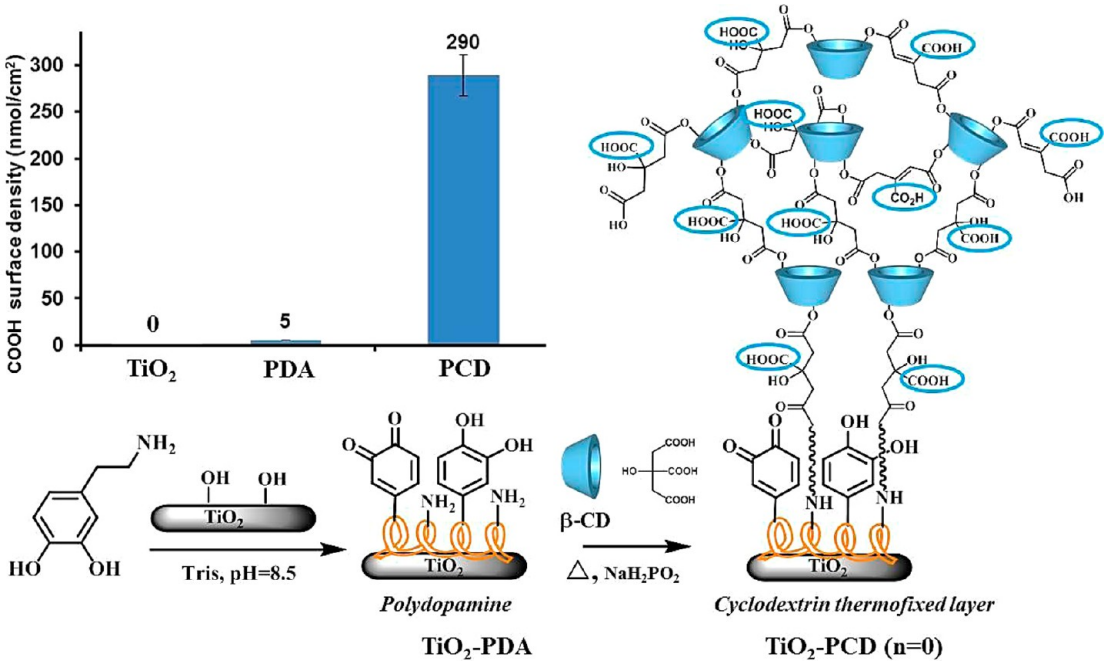

Table 1. Determination of the Coated Layer Thicknesses by Ellipsometry or Profilometry

method		ellipsometry			profilometry		
surfaces	TiO_2–PDA	n = 0	n = 0.5	n = 1	n = 5	n = 10	n = 15
thickness	64 ± 3 nm	60 ± 1 nm	76 ± 4 nm	92 ± 4 nm	1.5 μm ± 0.5	4 μm ± 1	7 μm ± 1

building block for modifying biomaterial interfaces. In particular, dopamine has sparked great interest as an anchor for the functionalization of titanium oxide surfaces due to the stability and strength of the resultant 5-membered metallocycle chelate.[59] In alkaline conditions, dopamine leads to the formation of a thin PDA film that exhibits latent reactivity toward nucleophiles and allows the anchorage of a plethora of biomolecules rich in amine or sulfhydryl groups.[51] Furthermore, PDA exhibits interesting biological properties including biocompatibility and hemocompatibilty and could play a pivotal role in cellular recruitment.[60] Recently, we have developed an original surface modification approach using the strong adhesive features of PDA to graft a cyclodextrin-based polymer layer onto metallic surfaces.[45] In this system, the role of the PDA sublayer was to promote the interactions between the metallic surface and an anionic PCD in situ generated from the PDA-modified surface through a polycondensation reaction (Scheme 2). In the present work, this grafting process was extended to the modification of titanium surfaces with a β-CD-based polymer (Scheme 2), and the resulting thermofixed CD layer was used as a first anionic layer for the building of the multilayer assemblies.

The grafting of PDA onto titanium surfaces was achieved by the immersion of titanium disks (ϕ = 1.49 cm) into a 2 mg/mL dopamine Tris buffer solution at pH 8.5 for 24 h. Afterward, the PDA-modified titanium plates were subjected to a thermal treatment at 160 °C to ensure the further attachment of the CD-based polymer[45] and to improve the biological properties of the coated surfaces.[61] For the PCD grafting, PDA-modified titanium samples were then immerged in a CTR/NaH_2PO_2/β-CD solution with a 10:3:10 ratio (g/100 mL of water) for 10 min at 37 °C (80 rpm) and were dried in a ventilated oven for 1 h at 90 °C. Thereafter, the polycondensation of β-CD and CTR was performed by curing at 140 °C for 30 min.

The formation of the PDA and the CD thermofixed layers was first investigated by colorimetric titrations (Scheme 2 insert). For this purpose, TBO was used as a probe because on the one hand it interacts with carboxylic groups through an ion exchange mechanism and on the other hand could be entrapped into the β-CD cavities due to its polycyclic and aromatic structure.[53] Colorimetric titrations results show that no carboxylic acid functions were observed on the titanium surface, whereas 5 nmol × cm^{-2} of the carboxylic acid functions, originating from the presence of pyrrolecarboxylic acid fragments in the polydopamine structure,[62] were observed for the PDA coating layer. The amount of carboxylic groups significantly increases to 290 nmol × cm^{-2} after the PCD grafting, thus indicating the strong anchorage of the cyclodextrin anionic thermofixed layer onto the titanium surface.

The PDA and PCD coatings on titanium surfaces were further characterized by ellipsometry analysis to evaluate the thickness of the grafting layers (Table 1 and Figure 2). The thickness of the PDA layer was measured at 64 ± 3 nm and is consistent with our previous results realized on CoCr surfaces and dealing with PDA coating.[45] Surprisingly, the coating layer thickness did not significantly change after subsequent functionalization of titanium surfaces with the thermofixed PCD layer. Nevertheless, a fit achieved with the ellipsometry fitting model based on sample structure revealed that the PCD film is entangled within the PDA layer and that the film thickness is 56 ± 1 nm ((free PDA = 4 ± 1 nm) + (PCD/PDA = 56 ± 1 nm)). These results are in accordance with previous

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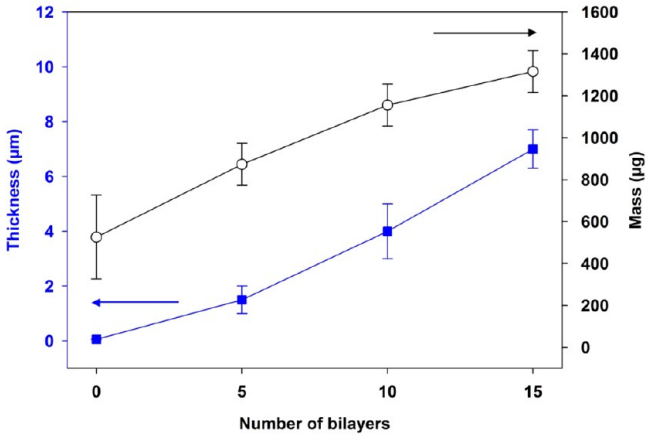


Figure 2. Evolution of the weight gain and the thickness of the titanium modified surfaces with the number of bilayers.

investigations realized by Zhao et al.[63] and dealing with PDA film behaviors. In this work, the authors claim that the PDA layer exhibits a membranelike porous nanostructure.

Elaboration of the Multilayer Assemblies. Once the titanium surface functionalized with the anionic CD thermofixed layer ($n = 0$), the polyelectrolyte multilayers were built up by alternating the dipping of the titanium PCD-functionalized samples into aqueous solutions (0.5% w/w) of positively and negatively charged polyelectrolytes, respectively (Scheme 3). According to the L-b-L deposition technique, charged polyelectrolytes were adsorbed onto the substrate surface through electrostatic interactions. In these studies, samples were functionalized with 5, 10, and 15 bilayers and labeled n = 5, 10, and 15 respectively.

Gravimetric Analyses. The functionalization of the titanium surfaces with the multilayer assemblies was proved by measuring the weight gain of the modified plates (Figure 2). This method allows us to control, in a simple way, the growth of the multilayer system onto the titanium plates and to estimate the amount of polymer immobilized after each grafting step. As observed in Figure 2, the weight gain increased almost linearly with the number of bilayers, suggesting that the formation of the L-b-L self-assembly takes place onto titanium surfaces and that the multilayers growth involves mostly the formation of strong polyelectrolytes pairs.

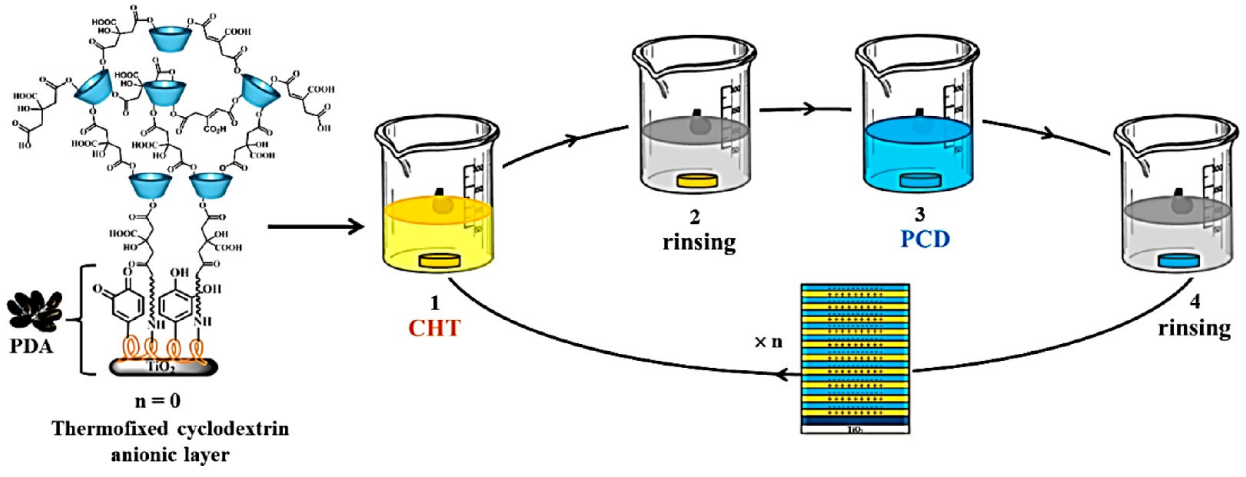
Colorimetric Titrations. The growth of the multilayer system was next investigated by colorimetric titrations to estimate the amount of both polyelectrolytes incorporated into the self-assemblies. For this purpose, TBO was used to evaluate the amount of CD-based polymer immobilized onto the titanium surface via COOH group quantification. OA, which complexes with ammonium groups of the immobilized CHT polymer in acidic media, was utilized to estimate the amount of CHT incorporated into the polyelectrolyte assemblies. As shown in Figure 3, TBO and OA content increased with the number of deposited bilayers, suggesting that the adsorption of PCD and CHT onto the titanium plates according to the multilayer growth is effective and that the number of cyclodextrin cavities available for drug storage increases with the number of bilayers.

SEM Investigations. The structure of the multilayer assemblies grafted onto the titanium surfaces was investigated by SEM analysis (Figure 4). For this purpose, a scratch was made with a Teflon tip on the titanium surface, and the samples were tilted to 45° to examine the morphologies of the coating layers and to estimate the film thicknesses. The presence of the multilayer systems onto the titanium surfaces is clearly evidenced because a coated layer is observed in each case and not in the case of unmodified titanium surfaces.

One can also note that the density of the grafted film increases significantly with the number of bilayers, thereby suggesting an enhancement of the cohesion within the hydrophilic polyelectrolytes in the film structure by increasing the number of bilayers in the PEMs system. From the SEM images, the film thicknesses were estimated to 1 ± 0.2 μm for n = 5, 3.5 ± 0.3 μm for n = 10, and 8 ± 1 μm for n = 15. Finally, analyses of the wall samples (see Figure S2 in the Supporting Information) indicate that the multilayer assemblies look uniform all over the sample, which is of prime importance in the context of medical applications.

Ellipsometry and Profilometry Measurements. The thickness of the first layers of the PEMs films was further characterized by ellipsometry measurements (Table 1). After the deposition of the first CHT layer onto the thermofixed CD anionic layer, the film thickness reached a value of 76 ± 4 nm. This value increased to 92 ± 4 nm after subsequent functionalization with PCD and resulted in a film thickness of around 15−16 nm for the first polyelectrolyte layers.

Scheme 3. Elaboration of the CHT−PCD Multilayer Systems from the CD Thermofixed Layer



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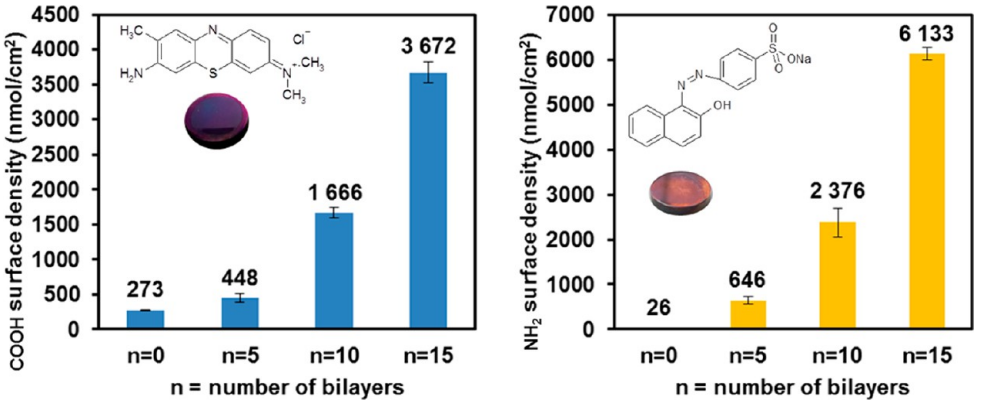

Figure 3. TBO and OA spectrophotometric titrations assessed on titanium surfaces during the building up of the multilayer assemblies.

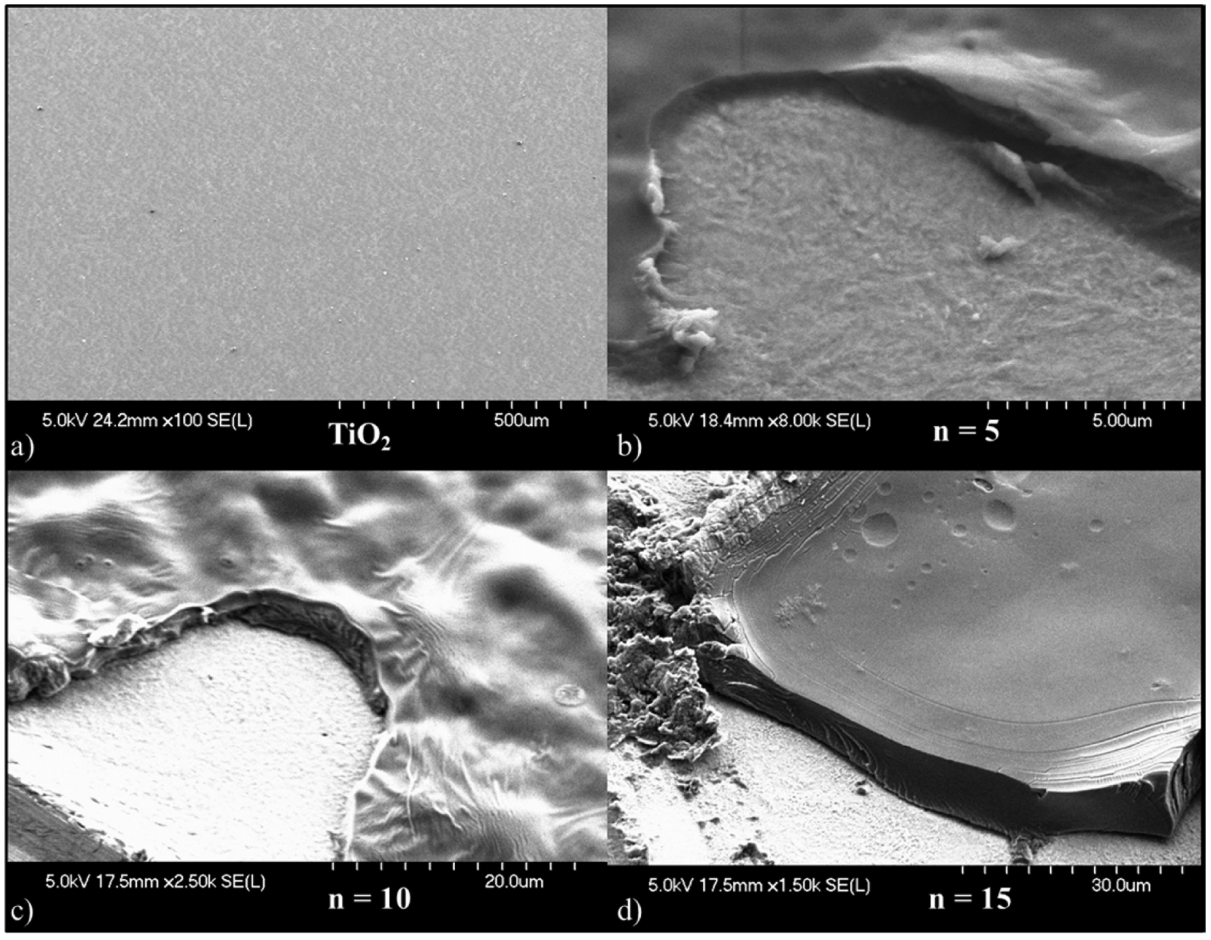

Figure 4. SEM images of (a) an uncoated titanium plate and (b–d) titanium plates functionalized with 5, 10, and 15 bilayers.

Unfortunately, thicker films could not be analyzed by ellipsometry because the light was scattered for the multilayers integrating a number of bilayers higher than $n = 1$. Subsequently, the film thickness for samples integrating $n = 5$, 10, and 15 bilayers was estimated by mechanical profilometry by scratching the films with a Teflon tip (Table 1 and Figure 2). The thickness was estimated at 1.5 ± 0.5, 4 ± 1, and 7 ± 1 μm for the multilayer assemblies containing 5, 10, and 15 bilayers, respectively, suggesting an almost linear growth of the thickness.

Degradation in Physiologic Conditions. The stability of the multilayer assemblies both in water and in physiological media was assessed by performing degradation studies on the system containing 15 bilayers. For this purpose, functionalized titanium plates were immersed in water and PBS solutions at 37 °C for 28 days. Afterward, TBO and AO titrations were performed to quantify the amount of carboxylic acid and amino functions remaining on the titanium plate surface (Figure 5). TBO quantification results showed that multilayer films were less degraded in water than in PBS solution after 10 days of immersion (Figure 5 insert). This can be attributed to the ionic strength of the salty PBS medium that may weaken the multilayer assemblies. Moreover, in the case of PBS medium, the carboxylic acid surface density of multilayer films decreased

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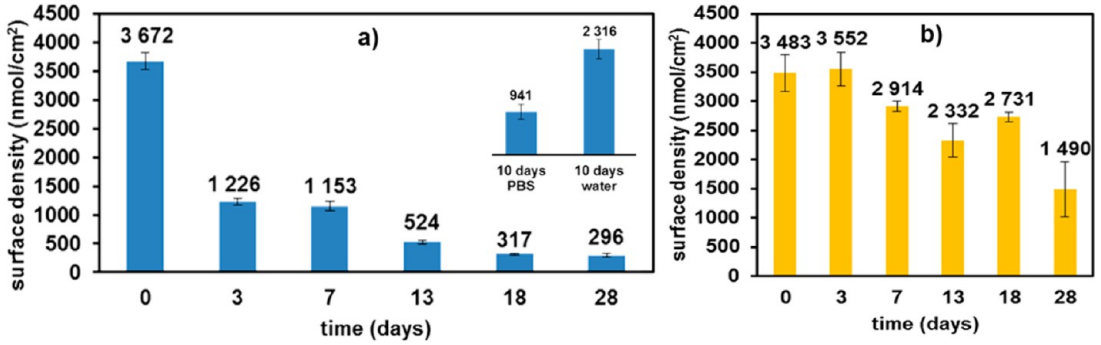

Figure 5. (a) TBO and (b) OA titrations realized on multilayer assemblies (n = 15) throughout the immersion in PBS medium.

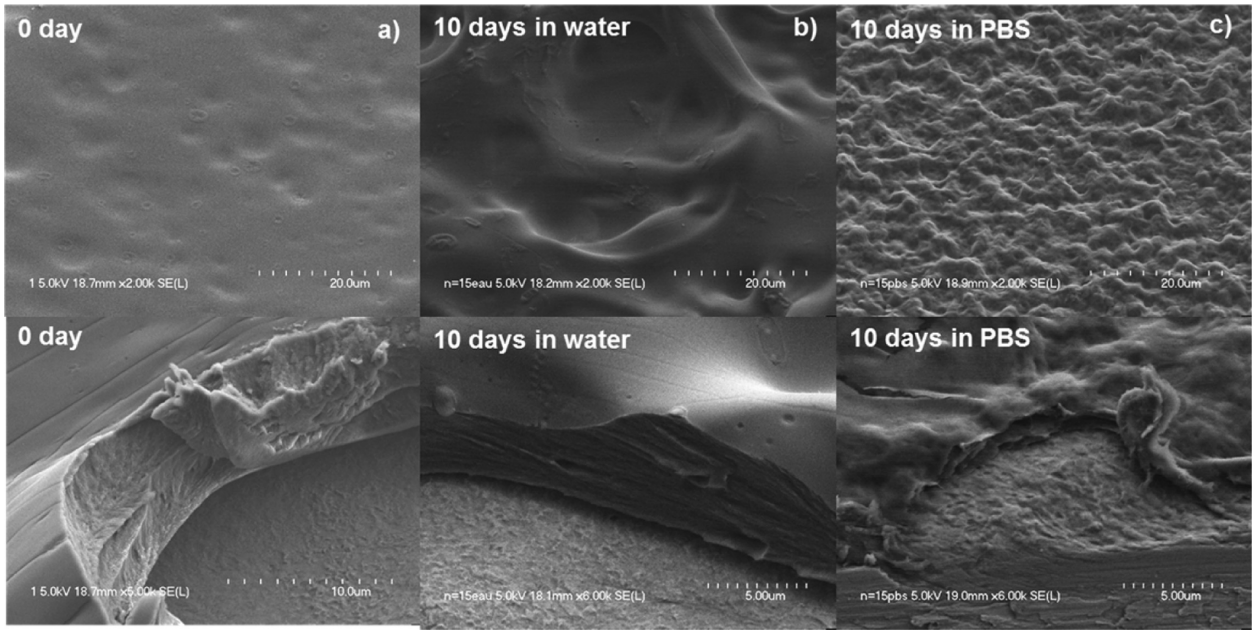

Figure 6. SEM images of (a) titanium plates functionalized with 15 bilayers and of the plates after 10 days of immersion in (b) water and (c) PBS.

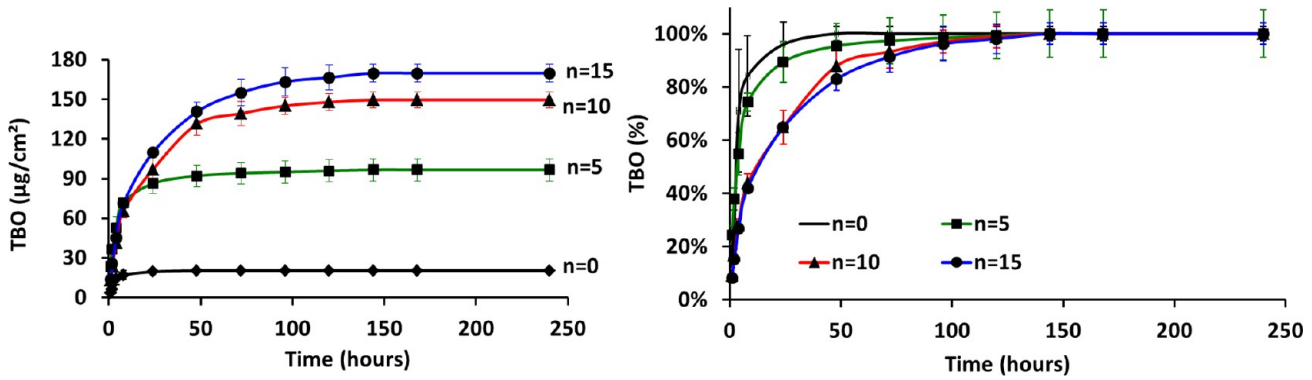

Figure 7. Release kinetics of TBO in phosphate buffer (pH 7.4, 37 °C, 80 rpm) from titanium disk samples functionalized with the multilayer assemblies.

dramatically with increasing the immersion time. Furthermore, 28 days were necessary to reach the surface density value (296 nmol × cm^{-2}), similar to that of the system when n = 0 (273 nmol × cm^{-2}), suggesting that the multilayer system was completely degraded after this period.

Surprisingly, this trend was not observed for CHT quantified through OA titrations. Indeed, despite a decrease of the cationic polyelectrolyte content with time, some CHT was still observed on the titanium surface after 28 days of immersion in PBS medium, suggesting that the multilayer degradation mechanism occurs probably by diffusion of the PCD within the multilayer assembly in the PBS medium. This result may be attributed to the better solubility of the PCD into PBS solution than CHT, which remains insoluble at pH 7.4.

The degradation of the multilayer system in PBS and water was further examined by SEM analyses (Figure 6). SEM images

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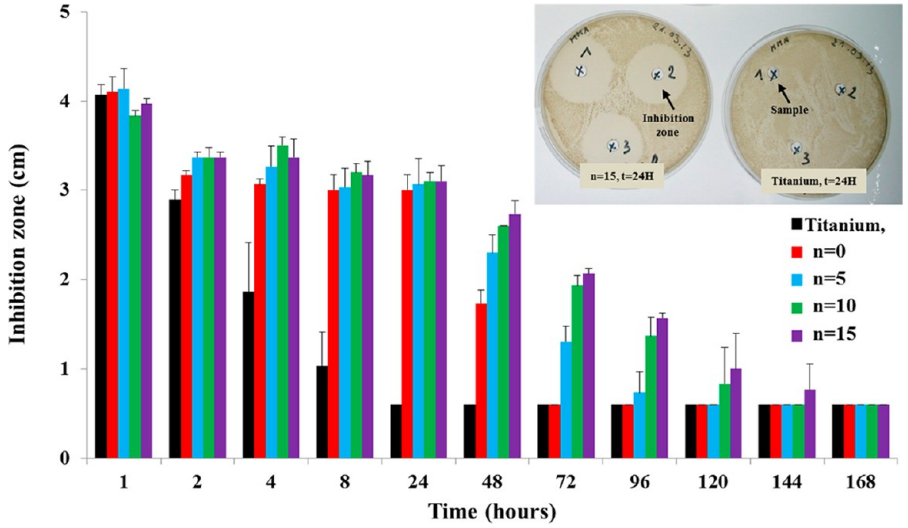

Figure 8. Antimicrobial activity of functionalized titanium disk samples loaded with gentamicin against *S. aureus*. Evolution of the size of the inhibition zone as a function of the contact time with PBS at pH 7.4 and T = 37 °C.

show the evolution of the surface morphology and thickness after 10 days of immersion at 37 °C in water and PBS. One can clearly observe that the PBS solution caused damages more quickly than water. Indeed, for each processing time, the surface morphology of samples immerged in PBS appeared less homogeneous than those immerged in water.

Furthermore, the observation of the coated layer after 10 days in PBS buffer shows that the thickness has dramatically decreased and that the self-assembled polyelectrolyte layer is affected. These observations are likely due to the degradation of the multilayer system through the disruption of the electrostatic interactions due to the ionic strength and the subsequent diffusion of the PCD within the multilayer film.

Release Kinetics. The ability of the titanium surfaces functionalized with the multilayer assemblies to absorb and release drugs in PBS medium was evaluated. For this purpose, TBO, which has a chemical structure very similar to that of antimicrobial methylene blue, was selected as a model drug to evaluate the release kinetic of each multilayer coating, as TBO offers the advantage of being entrapped in the β-CD cavity[53] and being easily quantifiable by UV−vis titrations. As depicted in Figure 7, the amount of released drug is very low for n = 0 compared to the amount in other systems, indicating the key role played by the multilayer system. Indeed, the amount of released drug increases with the number of bilayers as a result of the reservoir effect induced by the CD-based polymer. Hence, the multilayer assemblies prepared from PCD−CHT appear to be promising systems for the local controlled delivery of therapeutics. Furthermore, it can be observed that drug release rates increase from 1 day (n = 0) to 6 days (n = 15) with the increase of the number of bilayers constitutive of the multilayer system.

Finally, the relevance of the multilayer system in the conception of titanium surfaces targeting infections was evaluated with gentamicin, an antibiotic widely used in orthopedic surgery to treat or prevent infections. Our group has recently demonstrated that gentamicin could be immobilized onto PCD-based materials through ionic bonds without affecting the binding properties of CD moieties.[35] Therefore, this property offers the opportunity to incorporate a second drug within the biomaterial. Hence, gentamicin was loaded onto modified titanium by soaking samples in a 40 mg mL^{-1} solution of gentamicin for 4 h. After rinsing with water, the gentamicin-loaded disk samples (1 to 1.4 μg/cm^2) were soaked in PBS under shaking at 80 rpm and 37 °C and PBS renewal at regular intervals. Thereafter, the obtained PBS solutions were placed on *S. aureus* (CIP 224) preinoculated agar plates according to the Kirby−Bauer disk diffusion susceptibility test. Figure 8 shows the evolution of the microbial inhibition zone as a function of the contact time with the tested PBS solutions containing gentamicin. For the unmodified titanium plates, practically no microbial activity was observed after 24 h of incubation, indicating a fast release of gentamicin within the first day. On the contrary, an extended release of gentamicin was clearly observed with the coated multilayer systems; microbial activities were still observed after 6 days of incubation in the case of the multilayer system integrating 15 bilayers. It is noteworthy that the release kinetic as well the antimicrobial activity were improved by increasing the number of bilayers, indicating the key role of the multilayer system in the sustained release of gentamicin and in the antimicrobial activity of modified titanium plates. However, the degradation of multilayers into PBS solution is attributed most probably to the partial degradation of the PEMs through the diffusion of the CD-based polymer rather than the gentamicin diffusion phenomena.

CONCLUSIONS

New bioinspired titanium multidrug eluting platforms were elaborated through the self-assembly of CHT and a CD-based polymer, which acts as drug reservoir. For this purpose, an anionic thermofixed CD-based layer was first grafted onto titanium surfaces by exploiting the polydopamine film as an adhesive layer. Multilayer assemblies were then built up in mild conditions from the titanium platforms through electrostatic interactions according to the L-b-L strategy. The elaboration of the polyelectrolytes multilayers system (which alternates two biocompatible polyelectrolytes, i.e., PCD, as polyanion and CHT as polycation) was studied by means of SPR analysis, surface density determination, and weight gain studies. Ellipsometry and profilometry analyses were employed to estimate the thickness of the deposited layers, and the

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morphologies were assessed by SEM investigations. The efficiency of the L-b-L systems for adsorbing and releasing drugs in physiological conditions was validated with TBO as the drug model. Furthermore, the release kinetic studies revealed that, according to the reservoir effect of the CD-based polymer, the amount of loaded drug could be easily controlled by modulating the number of layers involved in the multilayer system. Finally, antimicrobial PEMs were prepared by loading the gentamicin drug, an antibiotic widely used to prevent infections. Antimicrobial investigations revealed that the functionalized surfaces exhibited a microbial activity up to 6 days of incubation with *S. aureus*, thus demonstrating the relevance of CHT−PCD PEMs in the conception of drug-eluting systems targeting infections.

Future works will focus on the exploitation of the ionic character of the PEMs system, as well the drug storage capacity of CD, with the aim to load at least two different and complementary drugs and provide multifunctional titanium platforms promoting the multiple therapeutic effect. A well-recognized strategy will involve the combination of two classes of antibiotics to address resistant germs[56,57] or the combination of antibiotics with anticoagulants[64] or analgesics.[65]

■ ASSOCIATED CONTENT

Supporting Information

SPR sensorgram (blank) and SEM images of functionalized titanium surfaces. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.5b02402.

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Notes

The authors declare no competing financial interest.

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