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Abstract

The stainless steel (SS) stents have been used in clinics since 1994. However, typical drawbacks are restenosis and thrombus formation due to limited endothelialisation and hemocompatibility. Surface modification is a smart strategy to enhance antithrombogenicity by promoting endothelialisation. In this work, the layer-by-layer (LbL) technique was applied for coating SS model substrates, after surface priming by functionalisation with 3-aminopropyl triethoxysilane (APTES). A LbL coating made of 14 layers of poly(styrene sulfonate)/poly(diallyldimethylammonium chloride) and heparin as last layer was deposited. FTIR-ATR analysis and contact angle measurements showed that LbL was an effective method to prepare nanostructured coatings. XPS analysis and colorimetric assay employing 1,9-dimethylmethylene blue dye to detect -COOH groups confirmed the successful polyelectrolyte deposition on the coated samples. Preliminary *in vitro* cell tests, using whole blood and human platelets, were performed to evaluate how surface modification affects platelet activation. Results showed that SS and SS-APTES surfaces induced platelet activation, as indicated by platelet spreading and filopodia formation. After surface modification by LbL coating, the platelets assumed a round shape and no fibrin nets were detected. Data demonstrated that LbL coating is a promising technique to fabricate antithrombogenic surface.

Introduction

316L stainless steel is widely used in coronary stent devices that have increased the quality and life expectancy of patients with coronary artery disease.[1] It provides an excellent combination of corrosion resistance with a broad range of mechanical properties. However, the exposure to flowing blood of the metallic stents can result in thrombus formation and smooth muscle cell proliferation, and ultimately lead to restenosis.[2,3] Currently, there is great demand for a new generation of coronary stents allowing rapid re-endothelialisation on the stent surface, which could provide protection against thrombus formation as well as minimise restenosis. Different approaches have been attempted to accelerate surface endothelialisation of stents, for instance stents have been pre-seeded with endothelial progenitor cells (EPCs)[4] or coated with anti-CD34 antibodies for the capture of patient's EPCs.[5] However, the use of biologically active substances make the production and sterilisation processes difficult; moreover, another limitation may lay in the relatively low content of the EPCs in blood.

The layer-by-layer (LbL) technique is a versatile solvent-free process allowing the coating of surfaces with uniform ultrathin multilayered films to tailor surface properties and structure at the nanoscale.[6] LbL is based on the alternating exposure of a charged substrate to solutions of positively and negatively charged polyelectrolytes. A rinsing step is included between each of the two previously described adsorption processes to remove excess material as well as to prevent cross-contamination of the polyelectrolyte solutions.[7,8] LbL approach is a promising tool to engineer interfaces, moreover its applicability to geometrically complex substrates provides a method for the control of cell adhesion on biomaterial substrates.[9,10] LbL is an alternative and simple approach for the preparation of functional surfaces with antithrombogenic properties and supporting endothelialisation for vascular tissue engineering and stent coating. Tan *et al.*[11] developed a multilayer film consisting of polyethylenimine (PEI) and heparin on biomedical 316L stainless steel surfaces via LbL technique. The result of *in vitro* tests of static hemocompatibility indicated that the biomedical coating significantly prolonged the static clotting time and reduced platelet adhesion. They demonstrated that electrostatic self-assembly of PEI/heparin on stainless steel stents resulted in non-thrombogenic coating. Lin *et al.*[12] prepared polyelectrolyte multilayers of antithrombogenic heparin and cytocompatible collagen in order to coat stainless steel stents, assuming that synergic properties of antithrombogenicity (deriving from heparin) and cytocompatibility (attributed to collagen) can be simultaneously conferred to the surface. They found that the number of the platelets on the LbL coated stents was significantly decreased and the cytocompatibility was greatly improved. Thus, the functional properties of the polyelectrolytes were combined in the coating.

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Key words: Stent endothelialisation; Surface modification; Silanisation; Stainless steel stent; Layer-by-layer.

Contributions: IC and VC were involved in the conception and the design of the work; IC and TN prepared samples, analysed and interpreted the data for what concerns physico-chemical characterisation; FB performed the biological tests.

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However, stable application of LbL coatings on implantable devices generally requires an initial surface priming treatment. 3-Aminopropyl triethoxysilane (APTES) is commonly used to functionalise inorganic substrates because it can form an amine-reactive film that is tightly attached to the surface. Silanisation of solid surfaces with APTES has been widely used to prepare substrates, which may be functionalised by immobilising proteins, and to prepare selective absorbents or organic/inorganic hybrid materials. Chemically adsorbed silane on an inorganic surface provides a platform for further functionalisation.[13] Silane film morphology can affect the accessibility of reactive groups for subsequent coupling with reactive molecules. The understanding of the relationship between morphology and amino group accessibility may lead to a more effective APTES application. In this work, APTES film was deposited on stainless steel substrates to favour LbL

coating deposition. APTES coatings were prepared by immersion in aqueous-ethanol APTES solution, after stainless steel surface activation by exposure to an alkaline solution. Subsequently, LbL coatings were applied on the APTES-modified stainless steel surface, with the aim to confer antithrombogenic properties to the metal surface. The layer-by-layer technique was performed by using poly(sodium 4-styrene sulfonate) (PSS) as polyanion, poly(diallyldimethylammonium chloride) (PDDA) as polycation, and heparin (HE) as last deposited polyanion. The obtained coatings were characterised through physico-chemical analyses and *in vitro* cell tests. The developed multilayered coatings could be exploited to incorporate drugs/growth factors with the aim to favour re-endothelisation and to inhibit restenosis by a bioactive coating able to avoid thrombus formation.

Materials and Methods

Materials

316L stainless steel (SS) plates (2.5x1.0 cm^2) with 100 μm thickness were kindly supplied by Carbostent and Implantable Devices C.I.D. s.r.l. and used as model substrates for implantable cardiovascular devices. 3-Aminopropyltriethoxysilane (APTES) with molecular weight of 221.37 Da, heparin sodium salt from porcine intestinal mucosa (HE≥180 USP units/mg), poly(sodium 4-styrenesulfonate) (PSS) with $\overline{M_w}$ ~70,000 Da, and polydiallyldimethylammonium chloride (PDDA) with $\overline{M_w}$ ~250,000-300,000 Da were purchased from Sigma-Aldrich s.r.l (Milan, Italy). All reagents were purchased from Sigma-Aldrich S.r.l and used without any further purifications.

Surface priming functionalisation

SS plates were silanised using APTES (SS-APTES) according to the method described by Meng *et al*.[14] The cleaned stainless steel plates were activated by dipping in 1M NaOH solution for 15 minutes to expose hydroxyl groups, rinsed with abundant bi-distillated water and dried at room temperature. Then the SS plates were incubated in 2% (w/v) APTES solution in 1/1 (v/v) water/ethanol for 5 h at room temperature; then they were rinsed with ethanol and dried at room temperature for 24 h and at 80°C for further 16 h.

Layer-by-layer deposition

The KSV NIMA Dip Coater (Biolin Scientific, Finland) was used to deposit the LbL coating on SS-APTES plate surfaces. To obtain the multilayered coating, SS-APTES samples were alternatively immersed into solutions of polyelectrolytes with opposite charge. The polyelectrolyte solutions were prepared dissolving the polyanions (HE and PSS) and the polycation (PDDA) into distilled water with a concentration of 0.5% (w/v), lowering the pH to 4.5 by the addition of drops of 1M HCl. Acid pH was necessary for the protonation of amino groups on SS-APTES surfaces, thus allowing electrostatic interaction between the surface and the first polyanion layer. After each immersion into polyelectrolyte solution, samples were accurately washed in distilled water, in order to eliminate the polylectrolytes in excess. A total number of 14 layers of PSS/PDDA was deposited on SS-APTES plates and HE was applied as last layer of the coating. The coating characterisation was performed as a function of the deposited layer numbers, as described in the next paragraphs.

Characterizations

SS-APTES samples were analysed under a fluorescence microscope (Zeiss Axiovert 100 M microscope, Zeiss, Oberkochen, Germany) equipped with a high-resolution digital camera controlled by AxioVision software (Zeiss). Photomicrographs were taken using a 100 W mercury arc light source lamp with a standard fluorescein (495 nm excitation/517 nm emission) and with A-plan objectives.

Atomic force microscopy (AFM) analysis was performed in true non-contact (NC) operational mode to avoid sample damage due to the softness of the investigated layers. μMasch silicon cantilevers, with a resonance frequency of 350 kHz and nominal radius smaller than 10 nm were used.

Static contact angles were measured at room temperature using a CAM 200 Instrument (KSV NIMA, Biolin Scientific, Finland) equipped with an Attention Theta software (Biolin Scientific, Finland) for data acquisition. Sessile drop method was applied, using a 5 μL Milli-Q water droplet.

The elemental chemical surface composition of functionalised SS plates was determined by XPS analysis using a VersaProbe PHI 5000 spectrometer (PHI Physical Electronics, USA) operating with a soft x-ray AlK source. XPS analysis was carried out in angle-reserved mode which analysis depth is 8 nm. PHI Summitt XPS was used as data acquisition software, while MultiPak XPS was used for data analysis. For each sample, O 1s, N 1s, C 1s, and Si 2p core levels were collected. All core-level peak energies were referenced to the saturated hydrocarbon peak at 285.0 eV.

For the determination of the density of sulfate groups, indicating the presence of PSS and HE on functionalised and control SS plate surfaces, a colorimetric protocol was applied. Functionalised and control SS plates were dipped into Taylor's Blue solution, prepared according to the protocol described by Rider,[15] for 1 hour and 30 minutes. Sulfate groups in heparin molecules link 1,9-dimethylmethylene molecules, so that functionalised samples turn from white to blue. 1 mL of the dissociating reagent, containing 3M of Guanidine hydrochloride (GuHCl) in phoshate buffered saline (PBS) at pH 7.5, was added to each sample with the aim to induce the release of the bound 1,9-dimethylmethylene dye molecules and heparin sulfate groups. After 30 min, dissociating reagent absorbance at 653 nm was measured using an UV-vis spectrophotometer Lambda 40 (Perkin Elmer, Italy). Measured absorbance was set against dissociation reagent for the blanks, calibration curve standards and test samples. Sulfate group concentration on functionalized samples was calculated through a calibration curve.

In vitro cell tests

In vitro tests were performed on SS, SS-APTES and SS-APTES-$(PSS/PDDA)_{14}$-HE, using whole blood. Samples were treated overnight in antibiotic cocktail (penicillin 50 U/mL, streptomycin 50 μg/mL and fungizone 2.5 μg/mL) solution (PBS). Native whole blood was collected from healthy male donors (males and females, age ≤60 years). A 50 μL drop of human platelets or whole blood was placed on the samples and incubated for 30 min at 37°C. Samples were withdrawn, then fixed in Karnowsky solution and dehydrated with graded ethanol (from 50 to 100% v/v), soaked for 30 min in hexamethyldisilazane, dried, and sputter-coated with Au. Samples were analysed by scanning electron microscopy using a LEO 1430 VP (Zeiss).

Results and Discussion

Surface priming functionalization

Surface priming functionalisation of stainless steel substrates with APTES was previously studied by our research group.[16] In this paper, the same method was used to introduce amino groups on SS plates. Briefly, in aqueous ethanol solution, APTES is hydrolysed and silanol groups are formed. The adsorption on the SS plates takes place via hydrogen bonding between surface hydroxyl groups and the silanol terminal groups of APTES molecules. If the concentration of surface hydroxyl groups is sufficiently high, APTES adsorbs mainly as isolated monomers. Multiple bonding to the surface is possible and should favour a molecular orientation where the alkyl chain is aligned vertically.[17,18] On the other hand, if the concentration of surface hydroxyl groups is low, APTES forms only occasionally bonds with the surface and its molecular orientation is more random.[19] Such molecules retain many free

silanol groups and can undergo vertical polymerisation by growth of siloxane chains. This type of bonding is observed as the formation of thick APTES clusters and can occur even at room temperature. Covalent bonding of the APTES films to the surface, i.e. the conversion of hydrogen bonds to Si–O–Si bonds, takes place mainly upon heating. During the stage of heating, lateral polymerisation between adjacent molecules of monolayered APTES coating, where unreacted silanol groups are still in abundance, is possible.[20]

Figure 1a and 1b shows images of the intrinsic fluorescence of SS and SS-APTES plates. Clean metal substrates were dark when observed under a fluorescence microscope; the fluorescence intensity of SS-APTES plates suggested the formation of a 3-dimensional film, as the APTES fluorescence was a result of a three-dimensional silane structure on the substrate.[13] As the AFM technique is particularly suitable for detecting either topographical inhomogeneities or material inhomogeneities at the sample surface, this technique was used to analyse surface topography. Topographic images of SS-APTES substrates showed the presence of particle agglomerates with submicrometer diameter and variable heights (Figure 1c). AFM phase images confirmed the continuous coating of substrates with APTES, as demonstrated by Figure 1d. XPS analysis was carried out on SS and APTES functionalised SS plates, to get an insight into the chemical composition of the coating. XPS spectrum (Figure 1e) showed a peak centred at around 400.0 eV due to nitrogen atoms of the NH_2 groups in APTES. Using peak deconvolution, a shoulder at 400.4 eV was evidenced, ascribed to positively charged nitrogen in the NH_3^+ form.

Nanostructured multilayer construction via LbL method

SS plates functionalised with APTES were coated with alternate layers of PSS and PDDA through the layer-by-layer deposition technique up to a maximum of 14 layers. HE was then deposited as the last layer in order to increase the hemocompatibility and with the future aim to facilitate electrostatic functionalisation with peptide sequences able to promote selective endothelial cells (ECs) adhesion, migration and proliferation on inner stent wall. HE has the highest negative charge density of any known biological macromolecule, due to its high content of negatively charged sulfo and carboxyl groups. The specificity of its interactions with a broad range of biologically important proteins is due to the defined pattern and orientation of sulfo and carboxyl groups able to promote specific protein interactions.[21] Moreover, PSS and PDDA were selected because they are both biocompatible and FDA approved.

The presence and the correct growth of the multilayered coating were detected through the measurement of static water contact angle and FTIR-ATR analysis after each deposited layer; moreover, the amount of deposited HE on the substrate surface was measured via a colorimetric method.

The static contact angle values of the pure polyelectrolytes were similar (53.0°±4.7° for PSS, 54.0°±1.9° for PDDA and 51.0°±5.9° for heparin) (Figure 2).[10] The contact angle value of SS coated with 2 bilayers increased due to the increase of surface roughness caused by polyelectrolyte islet formation. The contact angle was not significantly different from that of SS-APTES, for substrate coated from 5 to 10 layers. For a number of deposited layers higher than 10, contact angle progressively reduced at each layer deposition, reaching 35.1°±5.0° when last HE layer was deposited. To evaluate changes in the chemical characteristics of the surfaces of coated SS plates, FTIR-ATR analysis was performed on pure polyelectrolytes, SS plates, SS-APTES plates, SS-APTES-$(PSS/PDDA)_n$ and SS-APTES-$(PSS/PDDA)_{14}$-HE (Figure 3). Typical absorption bands of polyelectrolytes are reported in Table 1. The FTIR-ATR spectrum of PDDA[22] showed the band around at 3400 cm^{-1}, which is ascribed to water absorption and peaks at 2947, 2865, 1635, 1473, and 1418 cm^{-1} attributable to polymer skeletal vibrations. The FTIR-ATR spectrum of HE[23] showed the characteristic peaks at 3450 cm^{-1} assigned to hydroxyl stretching, at 2960-2860 cm^{-1} due to CH, CH_2 and CH_3 stretching, at 1650 cm^{-1} due to C=O stretching, and at

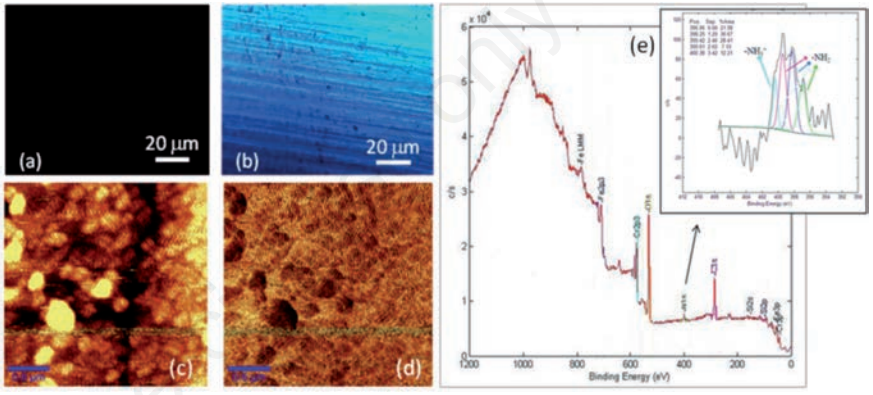

Figure 1. Fluorescence microscopy images of SS (a) and SS-APTES (b) plates. NC-AFM topographic (c) and phase (d) images of SS-APTES plates. (e) XPS spectrum and high resolution N1s spectrum of SS-APTES plates. Reproduced with permission from ref. 16. Copyright 2011, NSTI.

Table 1. Main absorption bands of polycations and polyanion in 1400-600 cm^{-1} wave number range.

Band (cm^{-1})	Assignment
PDDA	
3400	O-H stretching of absorbed H_2O
2947, 2865	C-H asymmetric stretching of $-CH_3$
1635	O-H symmetric deformation
1473, 1418	O-H asymmetric deformation
HE	
3450	O-H stretching
2960, 2860	CH, CH_2 and CH_3 stretching
1650	C=O stretching
1230, 1040	$-SO_3$ symmetric and asymmetric stretching
PSS	
3440	O-H stretching of absorbed H2O
2925-2848	CH and CH_2 stretching vibration
1630	C=C stretching
1449, 1009	CH and CH_2 bending
1182, 1128, 1039	$-SO_3$ groups
837	Aromatic ring vibration

PDDA, poly(diallyldimethylammonium chloride); HE, heparin; PSS, poly(styrene sulfonate).

1230 and 1040 cm^{-1} attributed to $–SO_3$ asymmetric and symmetric stretching.[23] The characteristic FTIR-ATR peaks of PSS samples were found at about 3440, 2925, 2848, 1630, 1449, 1182, 1128, 1039, 1009 and 837 cm^{-1} wavenumber. The band at 3440 cm^{-1} is assigned to the absorbed H_2O. The peaks at 2925 cm^{-1} and at 2848 cm^{-1} correspond to stretching vibrations of CH and CH_2 groups of the polymer chain; while the peaks at 1449 and 1009 cm^{-1} are attributable to bending of the same groups. The peak at 1630 cm^{-1} is due to the stretching of C=C. The peaks s at 1182, 1128 and 1039 cm^{-1} can be assigned SO_3 groups. The peak at 837 cm^{-1} originates from the aromatic ring.

FTIR-ATR spectra of SS-APTES-$(PSS/PDDA)_{14}$ showed the typical absorption bands of the polyelectrolytes: the O-H asymmetric stretching of PDDA at 3444 cm^{-1} and the overlapped O-H symmetric deformation and C=C ring stretching due to PDDA and PSS, respectively. The spectra of SS-APTES-$(PSS/PDDA)_{14}$ and SS-APTES-$(PSS/PDDA)_{14}$-HE were similar. FITR-ATR technique analyses a surface layer with 2-5 μm thickness and therefore the spectra of the LbL coated sample probably included the absorption bands from all the deposited layers. For this reason, the absorption bands of HE were not detected.

Therefore, XPS analysis was carried out on SS plate, SS-APTES plate and SS-APTES-$(PSS/PDDA)_{14}$ and SS-APTES-$(PSS/PDDA)_{14}$-HE to evaluate any difference in the chemical composition of the coating. In Figure 4 the obtained XPS spectra are reported.

The SS-APTES plate was characterised by the appearance of N1s peak, suggesting the presence of the amino groups on the sample surface necessary for further LbL coating deposition. In the LbL coated sample spectra, the intensity of N1s peak was only slightly lower respect to that of SS-APTES (Table 2), due to the presence of PDDA onto the surface. Moreover, the XPS spectrum of LbL coated samples showed the presence of sulfur peaks, due to the deposition of the polyanions (PSS and HE). The Fe peaks were no longer detectable in the XPS spectra of coated samples and the Cr peak significantly decreased from ~6 to ~1% (Figure 4c and 4d), suggesting complete coating of the surface and/or sufficient thickness. Table 2 reports the semi-quantitative analysis of the chemical elements present on the surface of samples measured by XPS, confirming the successful deposition of polyelectrolytes on the stainless steel substrates. The higher S percentage of SS- APTES-$(PSS/PDDA)_{14}$-HE confirmed the functionalisation with HE.

The quantification of sulfonate groups was carried out by incubating the samples in the Taylor's Blue solution: the dye molecules are able to electrostatically interact with the sulfonate groups present on the sample surface. By adding a 3M GuHCl solution at pH 7.5, the 1,9-dimethylmethylene molecules were released from the sample surface to the solution. The dissociating solution was then analyzed by UV-Vis spectrometer at 653 nm wavelength.

To relate the absorbance of the dissociating solution to the concentration of sulfonate groups, a calibration curve was obtained and was expressed by the following equation:

$$y=0.2199x$$

where the ordinate is the absorbance and x is the concentration of sulfonate groups (mg/mL). The sulfonate group concentration was evaluated on SS plates, SS-APTES-$(PSS/PDDA)_{14}$ and, SS-APTES-$(PSS/PDDA)_{14}$-HE. As shown in Figure 5, the sulfonate group concentration increased when the LbL coating was deposited onto sample surface: in fact, both polyanion molecules (PSS and HE) contain sulfonate groups. Moreover, the graph of Figure 5 shows that there was no significant difference in the sulfonate group concentration between the samples coated with 14 and

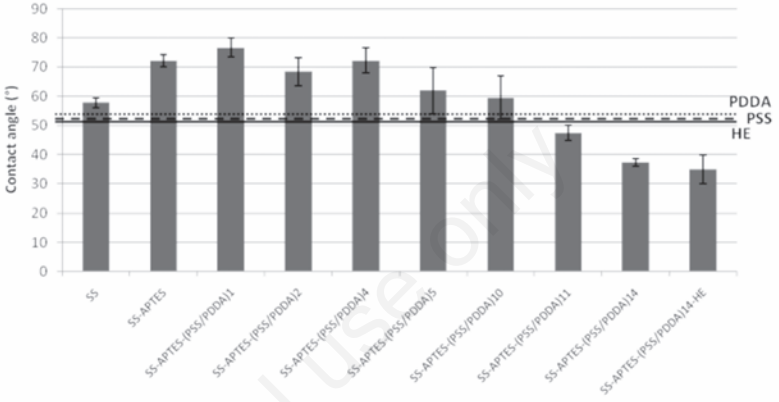

Figure 2. Static contact angle values of SS plates, SS-APTES, SS-APTES-(PSS/PDDA)n-HE (with n = 1, 2, 4, 5, 10, 11, 14) and SS-APTES-$(PSS/PDDA)_{14}$-HE. Data are average values and bars represent standard deviation. Horizontal lines represent the static contact angle values for PDDA (····), PSS(– – —) and HE (——) polyelectrolytes.

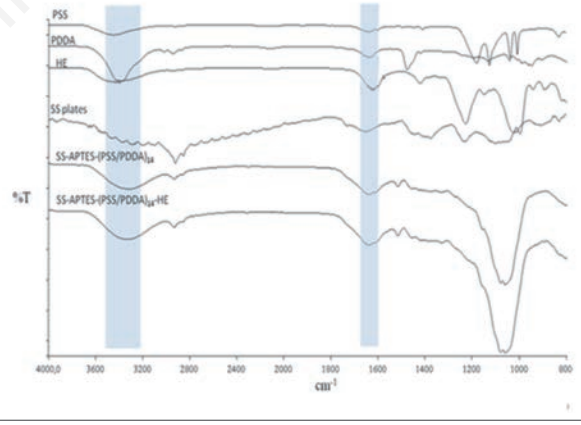

Figure 3. FTIR-ATR spectra of PSS, PDDA, HE, SS plates, SS-APTES-$(PSS/PDDA)_{14}$ SS-APTES-$(PSS/PDDA)_{14}$-HE.

Table 2. Atomic percentage of chemical elements present on the first atomic layers of analysed samples detected by X-ray photoelectron spectroscopy.

Sample	C1s (%)	O1s(%)	N1s (%)	S2p (%)	Cr2p3 (%)	Fe2p3 (%)
SS	51.4	39.2	-	-	5.6	3.8
SS-APTES	45.0	38.8	4.6	<0.1	6.9	4.7
SS-APTES-$(PSS/PDDA)_{14}$	73.8	18.0	4.2	2.7	1.3	-
SS-APTES-$(PSS/PDDA)_{14}$-HE	69.3	21.0	3.7	4.0	1.9	-

SS, stainless steel.

15 layers. Probably the colorimetric method measured the quantity of sulfonate groups in the whole multilayer. As a conclusion, the colorimetric method for the quantification of sulfonate groups confirmed the successful deposition of the polylectrolytes via LbL deposition technique.

The contact of blood with a foreign material surface leads to a sequence of events resulting in protein adsorption, activation of complement system and clotting cascade and finally thrombus formation.[24] The adhesion and activation of platelets mainly depend on the surface nature of the substrate and the adsorbed proteins.[25] To assess minimally surface thrombogenicity, SS, SS-APTES and SS-APTES-$(PSS/PDDA)_{14}$-HE plates were incubated with whole blood and human platelets. SEM images of *in vitro* cell adhesion are reported in Figure 6. SS-APTES was the least hemocompatible, as indicated by platelet spreading and filopodia formation; moreover, red blood cells were entrapped in a fibrin net, indicating the formation of stable clots (Figure 6b). SS plates showed spread platelets, which support their activation (Figure 6a and 6d). After surface modification by LbL coating, red cells slightly adhered on the surfaces (while no fibrin nets were detected) and platelets showed a round shaped conformation, indicating that were attached on the substrate but they are not activated (Figure 6c and 6f).

Conclusions

Functionalised coronary stents should allow rapid re-endothelialisation of the stent surface, which could provide protection against thrombus as well as minimise restenosis. To this aim, in this work, stainless steel plates, as model of coronary stents, were functionalised through the layer-by-layer technique in order to provide an antithrombogenic coating. The functionalisation of SS plates was carried out using a two-step approach: a surface priming treatment to expose amino groups and LbL deposition. APTES was used to perform the priming of SS plate surface. The proposed APTES deposition method allowed the preparation of continuous coatings with decreased wettability as compared to SS plates. The fluorescence microscopy analysis suggested a three-dimensional structure of the silane based film on the substrate, which was confirmed by AFM analysis. XPS analysis showed an increase of amino group amount on the surface of SS plates treated with APTES. SS-APTES plates were modified by LbL with PSS/PDDA multilayer coating followed by a final deposition of HE on the exposed surface. FTIR-ATR analysis demonstrated the successful deposition of polyelectrolytes. Static contact

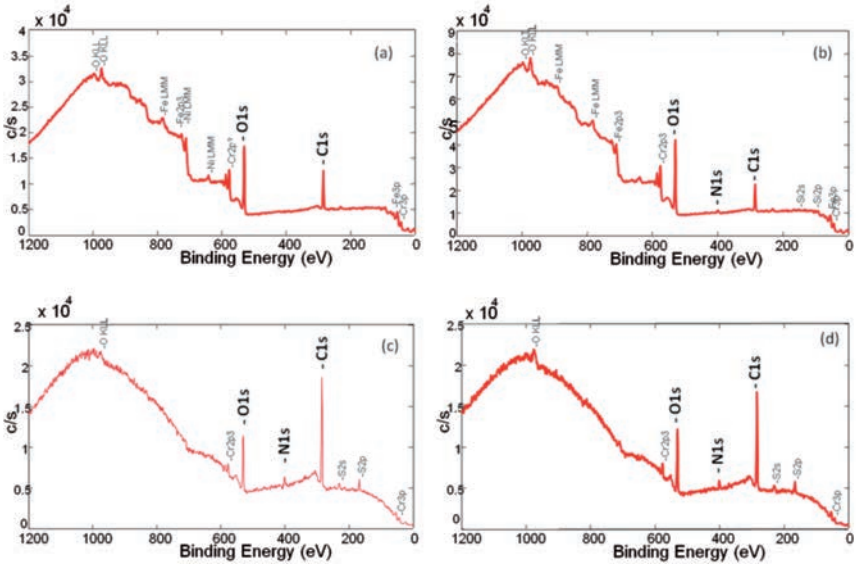

Figure 4. XPS spectra of: (a) SS plate; (b) SS-APTES plate; (c) SS- APTES-$(PSS/PDDA)_{14}$; (d) SS- APTES-$(PSS/PDDA)_{14}$-HE.

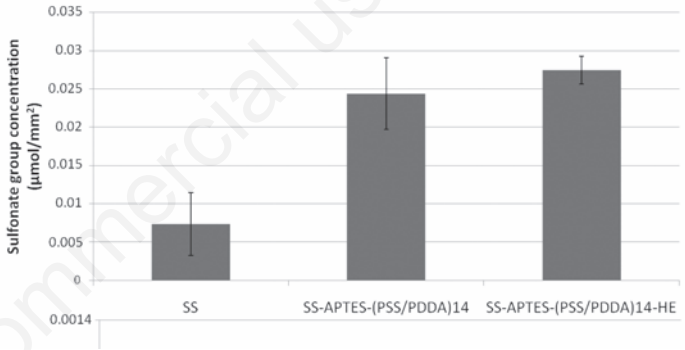

Figure 5. Sulfonate group concentration calculated from the absorbance of dissociating solution at 653 nm for the analysed samples.

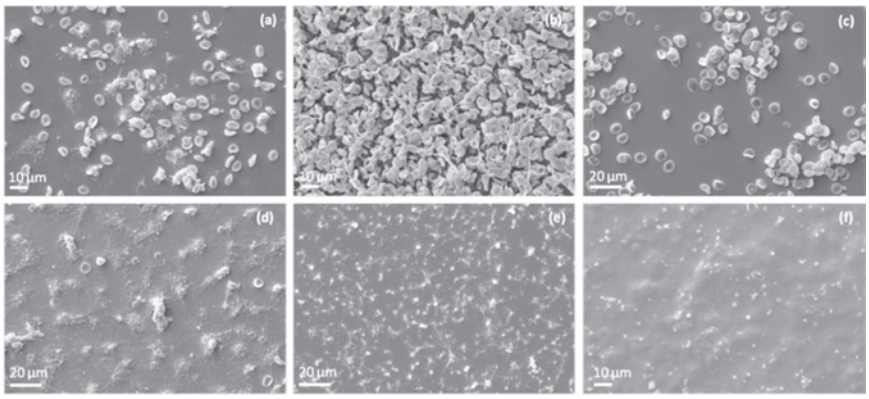

Figure 6. Scanning electronic microscopy (SEM) images of whole blood cells (a, b, c) on SS plates surface (a), SS-APTES surface (b) and APTES-(PSS/PDDA)14-HE surface (c). SEM images of human platelets (d, e, f) on SS plates surface (d), SS-APTES surface (e) and SS-APTES-$(PSS/PDDA)_{14}$-HE surface (f).

angles of coated samples progressively decreased with increasing the layer numbers, suggesting a more homogeneous deposition of the polyelectrolytes, particularly after 10 layer deposition [SS-APTES-$(PSS/PDDA)_{10}$-HE.] Colorimetric method employing Taylor's Blue for sulfonate group detection and XPS analysis confirmed the successful deposition of polyelectrolytes. The evaluation of platelet activation demonstrated that LbL coating inhibited the platelet adhesion and activation on stainless steel substrates.

These encouraging *in vitro* results make the layer-by-layer coating a promising flexible tool to obtain antithombogenic surfaces. In the future, LbL coating could be exploited for the surface functionalisation with bioactive peptides, able to electrostatically interact with HE, expositing selective bioactive recognition sequences for endothelial cell-surface interaction.

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