The surface modification of stainless steel and the correlation between the surface properties and protein adsorption

Chan-Koo Kang · Yoon-Sik Lee

Abstract Protein adsorption on a biomaterial surface is of great importance as it usually induces unfavorable biological cascades, with the result that much surface modification research has had to be performed in an effort to prevent this. In this study, we developed surface modification methods for stainless steel, which is a representative metal for biomedical device. The stainless steels were first smoothened to different extents by electropolishing, in order to obtain a rough or smooth surface. On these two kinds of substrates, we introduced epoxide groups to the metal surface by silanization with 3-glycidoxypropyltrimethoxysilane (GPTS). Then, various polymers such as poly(ethylene glycol) (PEG), poly(tetrahydrofuran glycol) (PTG), poly(propylene glycol) (PPG) and poly(dimethylsiloxane) (PDMS) were grafted on the silanized stainless steels. Each surface modification step was confirmed by various analytical methods. Contact angle measurement revealed that the surface hydrophilicity was controllable by polymer grafting. Root-mean-square (RMS) data of atomic force microscopy showed that surface roughness was dramatically changed by electropolishing. Based on these results, the correlation between surface properties and protein adsorption was investigated. In the protein adsorption study, we observed that all of the polymer-grafted stainless steels exhibited lower protein adsorption, when compared with bare stainless steel. Moreover, a hydrophilic and smooth surface was found to be the best of choice for decreasing the protein adsorption.

Keywords Stainless steel · Surface modification · Surface roughness · Hydrophilicity · Protein Adsorption

Introduction

Recent advances in biotechnology and material science are raising the interest in understanding of the effect of various materials on biological environment and developing materials without biofouling [1–4]. To improve the performance of biomaterials, a number of studies have been carried out in various fields such as materials design [5, 6], material composition [5, 7] and surface modification [8–10].

As a result of this multidisciplinary research, important advances are being made in surface modification design related to biomaterials. When implantable biomaterials are placed in a living body, their surfaces come into direct contact with host tissue or body fluids, which induces undesirable biological responses towards the foreign body such as protein adsorption, cell adhesion, thrombus formation, inflammation and immune reaction. The primary cause of these responses is protein binding on the biomaterial surface through interactions, such as electrostatic or hydrophobic forces. Non-specific protein adsorption triggers biological cascades that result in an abrupt loss of biocompatibility and functionality [1, 11]. Therefore, surface modification designed to generate a protein-resistant surface is gaining acceptance as a

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promising core technology in biomaterials development. However, surface modification has not yet been successfully achieved for all biomaterials and the relationships between the surface property of biomaterials and the biological responses that they induce, such as protein adsorption, are still poorly understood, although many attempts have been made to control these biological responses.

Surface modification of stainless steel is worth exploring because it is a material used in many biomedical devices. Stainless steel has been widely used in bone orthopedic implants, prostheses and vascular stents, due to its good corrosion resistance and excellent mechanical properties [12]. Despite these advantages, the surface of bare stainless steel is not compatible with biomedical applications. It occasionally causes unfavorable biological responses in the living body, when utilized without any surface treatment [13, 14]. Nevertheless, in contrast to other metals, such as titanium [15, 16], aluminum [17] and gold [18, 19, 28], as well as to silicon-based materials [20–22] and polymers [23, 24], reports on surface modification of stainless steel are few and far between, because it is difficult to functionalize its surface.

In order to design a biomaterial whose surface is rendered immune to biofouling via surface modification, it is important to understand how the surface property of a biomaterial affects protein adsorption on its surface. There are many factors that influence biocompatibility, however, protein adsorption on a biomaterial surface is of particular importance [25–28]. Surface roughness and surface hydrophilicity/hydrophobicity are also considered to be important factors [28–30]. In this study, to control the surface roughness, an electropolishing method was employed. Surface roughness control through electropolishing is more compatible with sophisticated biomaterials than other mechanical methods, because it is a non-contact and simple process. To adjust the surface hydrophilicity/hydrophobicity, polymer-grafting technology via covalent bonding was used. To bridge dissimilar materials such as stainless steel and polymers, a silane coupling agent was used [31, 32, 14]. Through silanization on a stainless steel surface, we introduced uniform functional groups, which could then be further reacted with preformed polymers, which are hydrophilic or hydrophobic. By changing the polymer's properties, such as its structure, molecular weight, backbone repeating unit and end terminal group, the surface of the biomaterial can be modified in various ways.

First, we demonstrated the surface modification of stainless steel via electropolishing and polymer grafting. Following this surface modification and characterization, we attempted to evaluate the dependence of the surface properties on protein adsorption by controlling the surface micro-roughness and hydrophlicity/hydrophobicity.

Specifically, the surface-modified stainless steels were characterized by contact angle goniometry, atomic force microscopy, X-ray photoelectron spectroscopy and confocal fluorescence microscopy. The amount of protein adsorption was evaluated by field-emission scanning electron microscopy and confocal fluorescence microscopy.

## Experimental procedure

Materials

AISI type 316L stainless steel plates with a thickness of 500 μm were purchased from Goodfellow Ltd., of Cambridge, UK. All of the plates were cleaned ultrasonically for 10 min in distilled water and acetone. Then, the stainless steel plates were cut into rectangles, 10×15 mm in size. 3-Glycidoxy-propyltrimethoxysilane (GPTS), diisopropylethyl-amine (DIEA), 4-(dimethylamino)pyridine (DMAP) and fluorescein isothiocyanate (FITC) were obtained from Aldrich and used as received. O,O'-Bis(amino-propyl)polyethylene glycol (PEG, Mn = 1,500 g/mol) was purchased from Fluka. The other polymers, polypropylene glycol bis(2-aminopropyl ether) (PPG, Mn = 2,000 g/mol), polytetrahydrofuran bis(3-amino-propyl) terminated (PTG, Mn = 1,100 g/mol) and polydimethylsiloxane bis(3-aminopropyl) terminated (PDMS, Mn = 2,500 g/mol) were purchased from Aldrich. A tetramethylrhodamine protein labeling kit was purchased from Molecular Probe. Human plasma fibrinogen was obtained from Sigma. All other chemicals were analytical grades and used without purification.

For the surface characterization and analysis, atomic force microscopy (AFM) (Digital Instrument, NanoScope IIIa), X-ray photoelectron spectroscopy (XPS) (PHI-5800, Physical Electronics), field-emission scanning electron microscopy (FE-SEM) (JEOL, JSM-6700F), static water contact angle measurement (SEO, Phoenix 300) and confocal fluorescence microscopy (Zeiss, LSM 5 Pascal) were used.

Surface modification of stainless steel

The overall surface modification scheme for stainless steel and the film structures are presented in Scheme 1.
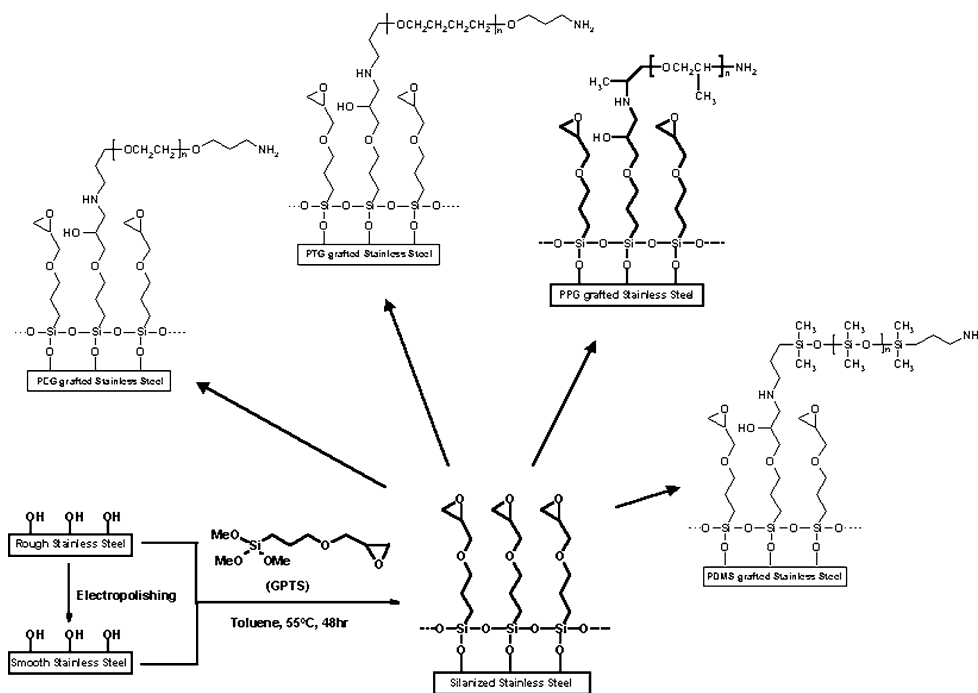
Scheme 1 Surface modification procedure of stainless steel

Electropolishing

To smoothen stainless steel surfaces in an electrolytic bath, a self-designed glass container was prepared. The stainless steel sample was linked to an anode, and a stainless steel reference plate was linked to a cathode. The distance between the sample and reference plate was 10 mm. In terms of the electrolyte conditions, we chose a solution of phosphoric acid, glycerol and water (34:47:19 wt%). The electropolishing time and current are 3 min and 1 A/cm^2, respectively. The settings of these two parameters are critical for obtaining consistent surface roughness. The electropolished stainless steel was rinsed copiously with deionized water and acetone.

Acid treatment and silanization

The stainless steels were acid-treated using a piranha solution composed of H_2SO_4 and H_2O_2 (4:1) for 1 h to remove any contaminants and expose the reactive hydroxyl groups on the surface. The acid-treated stainless steels were cleaned by ultrasonication in deionized water, ethanol and acetone for 10 min in a sequential manner, followed by nitrogen blowing, and were then immediately subjected to silanization. Silanization was performed with epoxy-functionalized silane (GPTS) in a 10% (*v/v*) GPTS/toluene solution at

55°C for 48 h. Subsequently, in order to eliminate the non-covalently adsorbed silane compounds, ultrasonication in toluene and methylene chloride was performed two times for 5 min each. The stainless steels were then dried gently under a steam of nitrogen gas and thermally cured in an oven at 70°C for 3 h. Prior to curing, purified argon gas was purged into the stainless steel containing vial to exclude all possibility of air oxidation.

Polymer grafting on the silanized stainless steel

In order to change the surface hydrophilicity by polymer grafting, we used four polymers, in which the backbone units were hydrophilic polyethylene glycol (PEG), meta-hydrophilic polytetrahydrofuran glycol (PTG), meta-hydrophilic polypropylene glycol (PPG) and hydrophobic polydimethylsiloxane (PDMS). Because these polymers were all of the diamine type, the epoxy-functionalized stainless steel surface could be reacted straightforwardly with the polymers under basic conditions. The grafting of the PEG, PTG and PPG polymers was carried out in a 10 mM polymer/NMP solution, into which six equivalents of DIEA with respect to the polymer were added. In the case of PDMS grafting, the same protocol was used, except that toluene was used as a solvent. Polymer grafting was performed in a shaking incubator

at 50°C for 24 h. After this, to eliminate any ungrafted polymer, ultrasonication was carried out sequentially in the grafting solvent and methylene chloride twice each for 10 min each time. The polymer-grafted stainless steels were dried gently using nitrogen gas and then stored in a vacuum oven until required.

Surface characterization of the modified stainless steel

Contact angle measurement

The static contact angle was measured on sessile drops, by taking the tangent to the drop on various stainless steel surfaces. Prior to the contact angle measurement, the stainless steel samples were fully dried in a vacuum oven for at least 24 h to exclude the effect of the washing solvent. Five contact angle measurements were made, each within 30 s after drop formation, and the results were averaged.

Atomic force microscopy

To detect the change in surface topography as modification reactions on the stainless steel surface proceeded, the bare, electropolished and polymers-grafted stainless steel surfaces were imaged by AFM tapping mode. Concurrently, surface micro-roughness was quantified for each surface-modified stainless steel. Micro-roughness analyses were performed at three random sites and the analyzed data were averaged. The scale of scanning images was 5×5 μm and z value was 100 nm.

X-ray photoelectron spectroscopy

XPS data was recorded using a monochromatized AlK_α source run at 15 kV and 24 mA. The spectra were recorded at take-off angles of 75°. The atomic ratios were determined by dividing each peak area by the corresponding sensitivity factor and subsequent normalization. Based on this, the elemental compositions (C, O, N, Si, Fe, Cr) of the acid-treated, silanized and polymers-grafted stainless steel surfaces were calculated.

Fluorescence analysis by confocal microscopy

Since the grafting polymers are all of the diamine type, the stainless steel surfaces were all amine-terminated as a result of polymer grafting. Thus, fluorescein isothiocyanate (FITC) was reacted with the aminated

surface and the FITC-coupled surface showed stronger fluorescence than the uncoupled surface. FITC coupling reactions on the bare and polymer-grafted stainless steel surface were performed in a 4 mM FITC/NMP solution at 30°C for 1 h, concurrently. After this, the stainless steels were ultrasonicated for 10 min in NMP and methylene chloride, sequentially, in order to remove physically adsorbed FITC, and then the fluorescence intensity was measured immediately by means of a confocal fluorescence microscope.

Biological characterization by protein adsorption assay

Fibrinogen adsorption study by scanning electron microscopy

Prior to the protein adsorption, the aminated polymer-grafted stainless steel surfaces were capped by acetylation, in order to exclude any electrostatic interaction between the amine groups on the surface and the proteins. Anhydrous methylene chloride (20 ml) and acetic anhydride (2 ml) were mixed, and then, 4-(dimethylamino)pyridine (1.0 mg) was added to the mixed solution. The polymer-grafted stainless steels were kept in the capping solution at 30°C for 24 h. After the capping, each sample was ultrasonicated twice for 5 min in methylene chloride and dried by nitrogen.

Human plasma fibrinogen was dissolved in a 50 mM phosphate buffer (pH = 7.4), to provide a final solution concentration of 0.5 mg/ml. For all nine samples, i.e. one bare stainless steel, four unelectropolished/polymer-grafted stainless steels and four electropolished/polymer-grafted stainless steels, protein adsorption was allowed to proceed in a shaking incubator for 2 h at 37°C. Following protein adsorption, all of the samples were thoroughly rinsed 10 times with phosphate buffer to remove any non-adsorbed proteins, and then washed five times with copious deionized water to remove the buffer salts. The samples were dried gently with nitrogen and imaged immediately by field-emission scanning electron microscopy (FE-SEM).

Quantification of adsorbed fibrinogen

To quantify the amount of adsorbed fibrinogen, we chose a fluorescence detection method, due to its simplicity and high sensitivity. For fluorescence detection, rhodamine succinimidyl ester was coupled to the fibrinogen. The degree of labeling of the fluorescence dye was approximately 4–5 molecules per fibrinogen.

The amine capping procedure, buffer system, solution concentration and adsorption study protocol were the same as those described in Sect. 2.4.1. Rhodamine–fibrinogen was applied to all nine samples as described in Sect. 2.4.1, and the proteins adsorbed on the stainless steel were detected immediately by means of a confocal fluorescence microscope. The scale of the observation images was 930 ×930 μm.

Results and discussion

Surface characterization

Surface hydrophilicity

The contact angle data of the surface-modified stainless steels are summarized in Table 1. The water contact angle data provides direct evidence of the change in surface hydrophilicity as the surface modification proceeded. The bare stainless steel surface was very hydrophobic and the contact angle was as high as 79°. However, after acid treatment, the hydrophobic bare stainless steel surface became highly hydrophilic, causing hydroxyl groups to become exposed on the surface, such that the silanization reaction was possible. After introducing the alkyl epoxide group through silanization with GPTS, the contact angle increased drastically from <10° to 61°. After the polymers were grafted on the silanized surfaces, the surface hydrophilicities were changed to varying degrees. Depending on the kind of polymers grafted on the surface, contact angles were either decreased or increased, with the change in contact angle ranging from 46° to 71°. Based on this data, we concluded that the polymers were successfully grafted on the stainless steel surfaces and that the surface hydrophilicity could be readily

controlled by polymer grafting. Both the rough (unelectropolished) and the smoothened (electropolished) surfaces yielded similar contact angle data, indicating that the surface modification proceeded in a similar manner, regardless of the surface roughness.

Surface topography and roughness

To investigate the surface-modified stainless steels and analyze the surface micro-roughness, we used AFM after each modification step. The surface topography images are shown in Fig. 1 and the micro-roughness values are summarized in Table 2. First, many of the hills and valleys, which were observed in the bare surface, disappeared after electropolishing. Accordingly, the root-mean-square (RMS) roughness decreased significantly from 116.4 to 1.4 nm. After silanization and polymer grafting on the electropolished stainless steels, some protrusions and many tiny peaks were detected, and the RMS data increased slightly from 1.4 to approximately 6–10 nm. Depending upon the polymer structure, the surface topography and RMS data differed slightly. These results indicated that the polymers were well grafted, and that the polymer-grafted surfaces were of the brush type [22]. In the case of the unelectropolished stainless steels, no major differences in surface structure were observed before and after polymer grafting, because the polymer grafting effect on the RMS data seemed to be too small.

Four kinds of polymers were grafted on both the rough or smooth surfaces, providing eight samples that possess various surface properties in terms of micro-roughness and hydrophilicity.

Elemental analysis

XPS measurements were made on three different surfaces, i.e. those of the acid-treated stainless steel, silanized stainless steel and polymer-grafted stainless steel. Table 3 summarized the normalized atomic compositions on each surface. All of the results shown in Table 3 were obtained from the electropolished stainless steels for the sake of consistency. As in the case of the previous results pertaining to the surface characterization, there were no major differences in the atomic compositions between the unelectropolished stainless steel and electropolished stainless steel after each modification step.

In Table 3, the carbon signal on the acid-treated stainless steel arose from the intrinsic carbon content and inevitable adventitious hydrocarbon contaminants. Likewise, the strong oxygen signal was attributed to

Table 1 Static water contact angles of the surface-modified stainless steels

Sample	Contact angle (°)
Bare stainless steel	78.8 ± 1.9°
Acid-treated stainless steel	<10°
GPTS-silanized stainless steel	61.4 ± 4.2°
PEG-grafted stainless steel	46.0 ± 4.0° (45.8 ± 3.8°)[a]
PTG-grafted stainless steel	52.6 ± 4.3° (52.2 ± 4.2°)
PPG-grafted stainless steel	54.0 ± 4.2° (53.4 ± 4.4°)
PDMS-grafted stainless steel	70.6 ± 4.2° (69.0 ± 5.2°)

[a] For the polymer-grafted surfaces, contact angle data on unelectropolished stainless steel surfaces and electropolished stainless steel surfaces were presented. Parenthesized data were contact angle on the unelectropolished stainless steel surfaces

Fig. 1 AFM images of the surface-modified stainless steels (5 × 5 μm, z = 100 nm). (**a**) Bare surface, (**b**) electropolished surface, (**c**) electropolished/PEG surface, (**d**) electropolished/ PTG surface, (**e**) electropolished/PPG surface, (**f**) electropolished/ PDMS surface
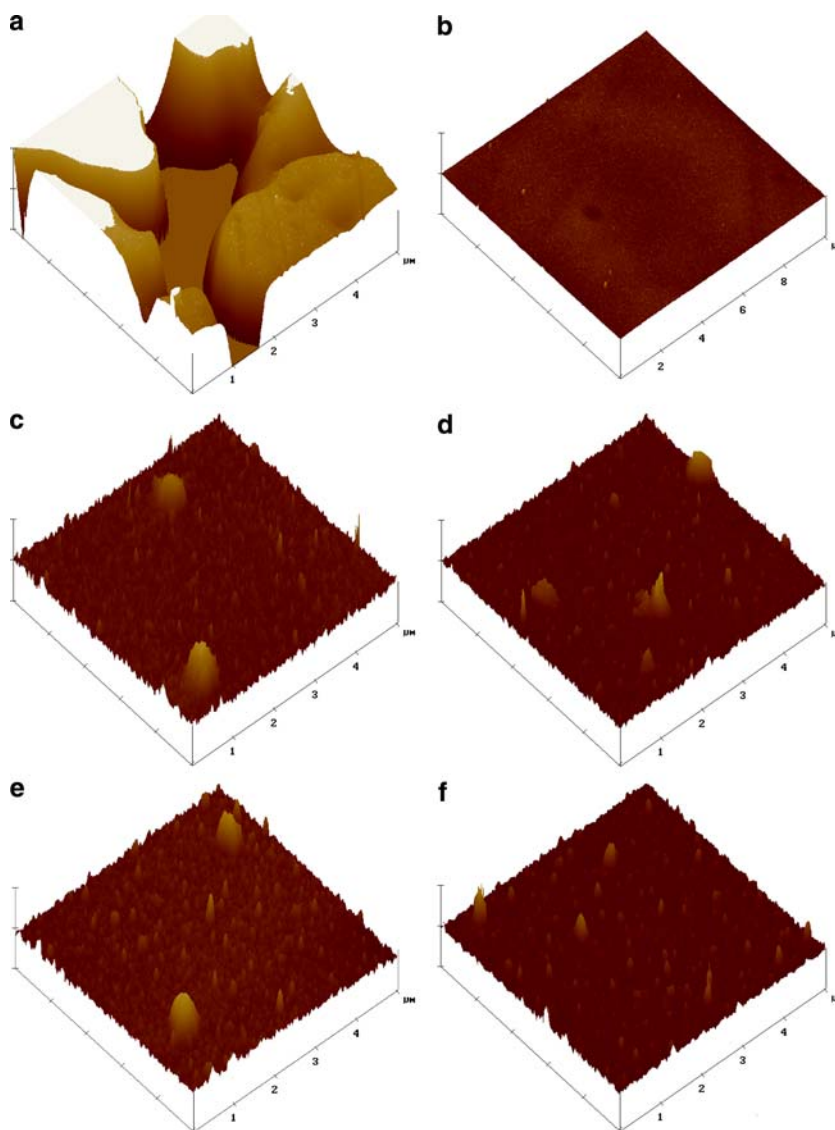

Table 2 Surface micro-roughness of the surface-modified stainless steels

Sample	Micro-roughness (RMS) (nm)
Bare stainless steel	116.4 ± 26.3
Electropolished stainless steel	1.4 ± 0.4
GPTS-silanized stainless steel	11.4 ± 2.1
PEG-grafted stainless steel	10.6 ± 0.3 (122.5 ± 11.0)[a]
PTG-grafted stainless steel	9.0 ± 2.3 (99.8 ± 12.1)
PPG-grafted stainless steel	8.6 ± 1.0 (110.9 ± 16.6)
PDMS-grafted stainless steel	5.6 ± 0.5 (119.8 ± 13.0)

[a] For the polymer-grafted surfaces, RMS data on unelectropolished surfaces and electropolished surfaces were presented. Parenthesized data were RMS on the unelectropolished stainless steel surfaces

the hydrated oxide coating of the stainless steel. The hydroxyl groups on the acid-treated surface almost certainly reacted with the silane compound, and this

was confirmed by the carbon content increase and metal contents decrease. The formation of a silane overlayer was also confirmed by the dramatic enhancement of the Si signal. On the polymer-grafted stainless steels, the increase of N composition was prominent, due to the amine terminal groups of the polymers. We also found that the carbon content increased more and the metal contents decreased after polymer grafting, when compared with the silanized stainless steel. This proved that the polymers were successfully grafted on the silanized stainless steel. The change in atomic composition confirmed that the planned surface modifications were achieved.

Fluorescence analysis for polymer grafting

Quantitative analysis of amine groups on a stainless steel surface by FITC conjugation can provide

Table 3 Atomic composition of the surface-modified stainless steels (at. %)

Sample	C(1s)	N(1s)	O(1s)	Si(2p)	Cr(2p)	Fe(2p)
Acid-treated stainless steel	19.3	2.1	48.2	1.8	17.0	11.6
GPTS-silanized stainless steel	36.5	1.7	43.4	6.1	8.3	4.0
PEG-grafted stainless steel	48.9	4.8	34.7	7.2	3.0	1.4
PTG-grafted stainless steel	53.1	4.0	33.5	6.0	2.2	1.2
PPG-grafted stainless steel	47.6	3.0	37.7	7.4	2.9	1.4
PDMS-grafted stainless steel	55.7	3.4	29.4	8.8	2.1	0.6

evidence as to whether the surface modification reactions have been accomplished or not. Fluorescence intensity data are presented in Table 4 for both the rough and smooth surfaces. As expected, the bare stainless steel showed no fluorescence, because it has no functional groups on the surface. However, all of the polymer-grafted stainless steels showed stronger fluorescence intensities than the bare stainless steel. The fluorescence intensities on the polymer-grafted stainless steels were uniform over each scan area. From this result, we can conclude that polymer chains were immobilized homogeneously and that the polymer grafting was not affected by the surface roughness.

Biological characterization by protein adsorption assay

Protein adsorption on a biomaterial surface usually occurs in a mixture of solutions containing many biomolecules and, thus, biological interactions at the surface are the overall result of complex cooperation, competition and interference between the biomolecules and the biomaterial. Because handling all of the mixed biological solutions and analyzing the individual interactions are very difficult, we chose fibrinogen as a

Table 4 Fluorescence intensity of the aminated stainless steel surfaces by polymer grafting

Sample	Fluorescence intensity (a.u.)	
	Rough (unelectropolished)	Smooth (electropolished)
Stainless steel	907.6 ± 96.8	894.9 ± 69.6
PEG-grafted stainless steel	>4,095[a]	>4,095
PTG-grafted stainless steel	>4,095	>4,095
PPG-grafted stainless steel	>4,095	>4,095
PDMS-grafted stainless steel	3883.2 ± 163.1	3130.0 ± 235.3

[a] In confocal fluorescence microscopy, the detection limit of fluorescence was 4,095 a.u.

model for the protein adsorption assay on the surface-modified stainless steels. Fibrinogen is a biochemical marker in biological cascades, such as thrombosis, and is occasionally used as a standard to evaluate the biocompatibility of biomaterial [33, 7].

SEM images and confocal fluorescence microscopy results for the adsorption of fibrinogen onto the surface-modified stainless steels are presented in Figs. 2–4. First of all, all of the surface-modified stainless steels showed considerably lower fibrinogen adsorption in both the SEM (Fig. 2) and confocal fluorescence microscope images (Fig. 3), as compared to bare stainless steel, which was used as a control. In Fig. 2, SEM images of the bare, unelectropolished/ PEG grafted, and electropolished/PEG grafted stainless steels are presented as representative examples. By means of confocal fluorescence microscopy, we found that the fluorescence intensities of the fibrinogen adsorbed on the surface-modified stainless steels were as much as 72–83% lower than those on the bare stainless steel surface (Fig. 4). This result is presumably due to the configurational entropy repulsion of the grafted polymers, preventing the protein from approaching the surfaces. The polymers used in this experiment were composed of simple linear chains and were very flexible. The entropy penalty associated with the compression and penetration of protein into the flexible polymer chains makes the polymer-grafted surface protein-resistant [26, 34].

To isolate the effects of micro-roughness and hydrophilicity on the surface of the protein adsorption from other factors, namely the entropy effect of polymers, the grafting density and the terminal group effect, we capped the terminal amine groups of the grafted polymers by acetylation. After capping, FITC was coupled as described in Sect. 2.3.4. The capped surface showed no fluorescence, thus confirming that complete capping was achieved. Regarding the entropy effect of the polymers, the approximate lengths of fully extended all-trans configuration of the polymers are considered to be ~122 Å for PEG, ~90 Å for PTG, ~124 Å for PPG and ~109 Å for PDMS. The entropy
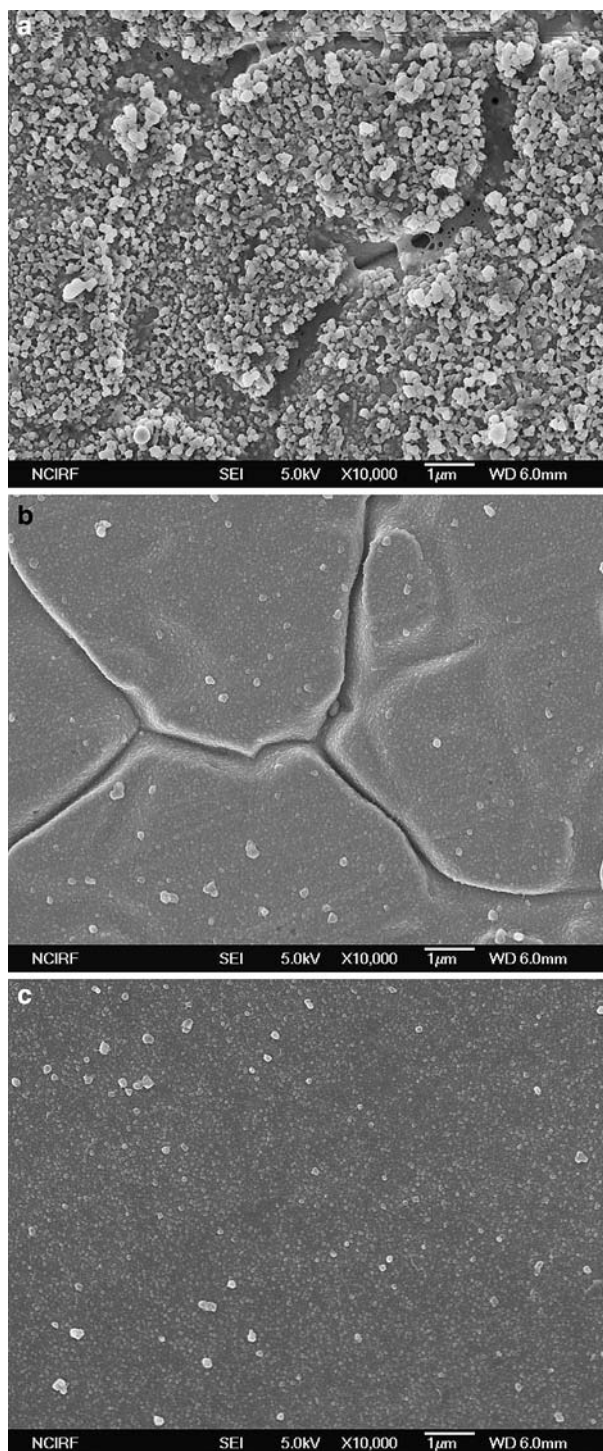
Fig. 2 Scanning electron micrograph images of protein adsorption to the surface-modified stainless steels. (**a**) Bare surface, (**b**) unelectropolished/PEG surface, (**c**) electropolished/PEG surface

effect inferred from these lengths is thought to be similar. Also, the polymer grating densities on the stainless steels were shown to be comparable, as inferred from their similar fluorescence intensities.
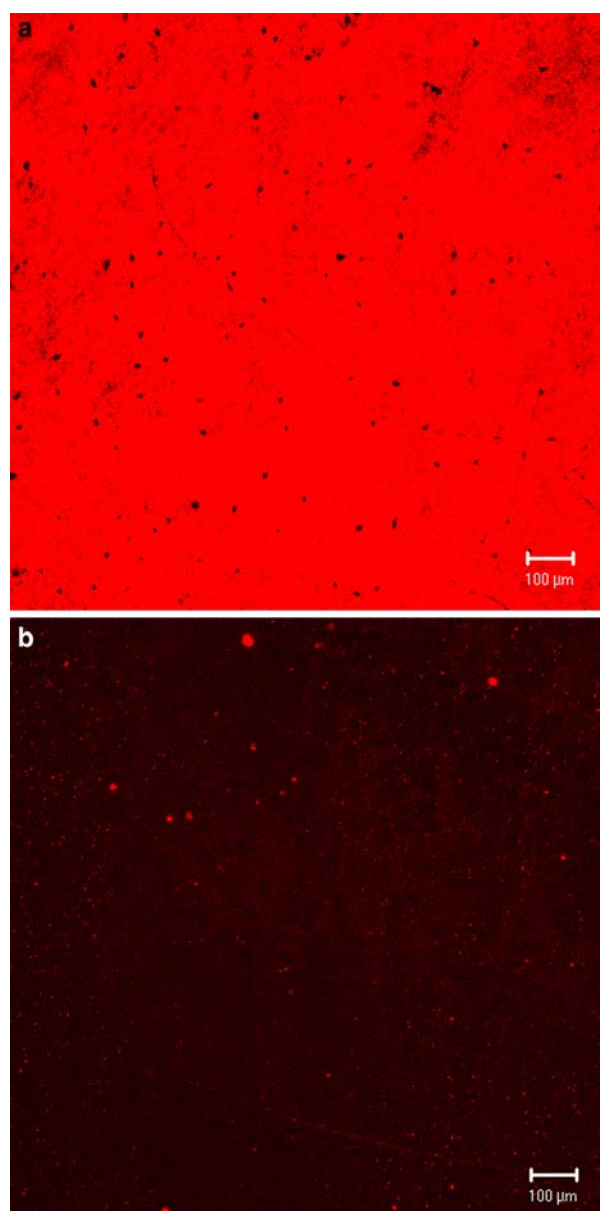

Fig. 3 Rhodamine–fibrinogen adsorbed images by confocal fluorescence microscope. (**a**) Bare stainless steel, (**b**) electropolished/PEG stainless steel

In order to investigate the correlation between the surface properties and the protein adsorption in detail, the images obtained using the confocal fluorescence microscope were analyzed. These results are summarized in Figs. 5, 6. The data shown in Fig. 5 indicate that the protein adsorption is closely related to the surface hydrophilicity. On the PDMS-grafted hydrophobic surface, fibrinogen molecules were adsorbed in larger amounts than on the less hydrophobic (PEG, PTG, PPG) surfaces. Compared with the PEG-grafted surface, which is the most hydrophilic, about

Fig. 4 Fluorescence intensity graph of the rhodamine–fibrinogen adsorbed stainless steel surfaces. (**a**) Bare surface, (**b**) unelectropolished/PEG surface, (**c**) unelectropolished/PTG surface, (**d**) unelectropolished/PPG surface, (**e**) unelectropolished/PDMS surface, (**f**) electropolished/PEG surface, (**g**) electropolished/PTG surface, (**h**) electropolished/PPG surface, (**i**) electropolished/PDMS surface
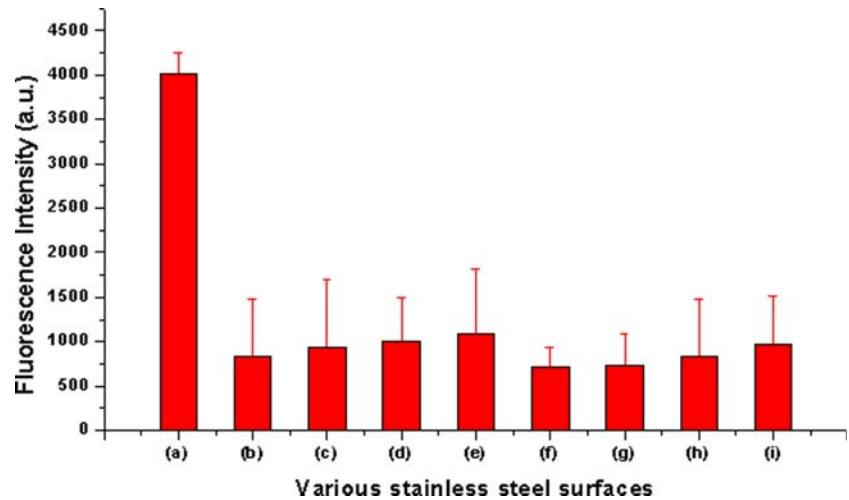
30% more fibrinogen adsorption occurred on the PDMS-grafted surface. It has been reported that water molecules situated between the proteins and the surface take part in reducing the protein-substrate attraction. The stability and thickness of the interfacial water layers largely affects the protein adsorption. Because water content at the surface increases on a hydrophilic surface, the result in Fig. 5 is consistent with those of previous reports [34, 35, 28]. The data shown in Fig. 6 demonstrate that there is a correlation between the surface micro-roughness and protein adsorption. Although the correlation tendency is weaker than that of the surface hydrophilicity, we found that rough surfaces consistently resulted in more protein adsorption. Specifically, the protein adsorptions on the electropolished/polymer-grafted surfaces were decreased by 4–9% compared with those on the unelectropolished ones. The surface area was also decreased by electropolishing. From this, we could conclude that the reduction in the surface area caused

by electropolishing gave rise to a decrease in the number of fibrinogen binding sites, which in turn led to less fibrinogen adsorption.

From these results, we could conclude that the more hydrophilic and smoother the surface is, the less protein adsorption occurs on the surface. The electropolished and PEG-grafted hydrophilic surface was the ideal surface for inhibiting protein adsorption, whereas the rough and hydrophobic surface showed the worst result. The difference in protein adsorption between the best and worst case was about 40%, and the effect of surface hydrophilicity was more critical, when compared to the surface micro-roughness.

Conclusions

In the present research, the surface modification of stainless steel was successfully achieved by electropolishing, silanization and the covalent attachment of
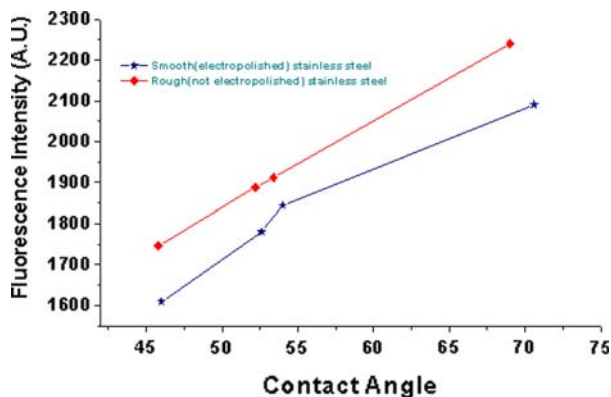

Fig. 5 Amount of the fibrinogen adsorption on the surface-modified stainless steels vs. corresponding water contact angle
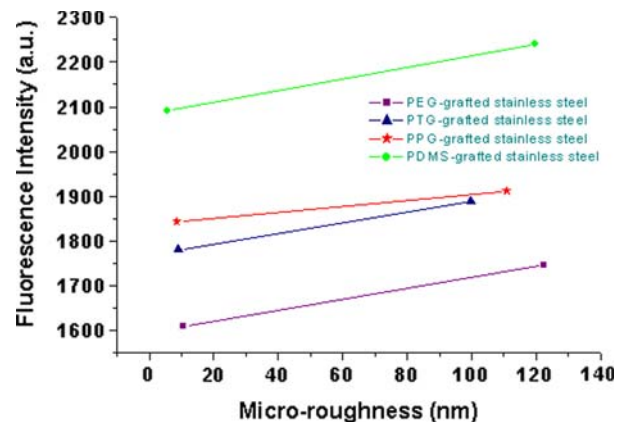

Fig. 6 Amount of the fibrinogen adsorption on the surface-modified stainless steels vs. corresponding micro-roughness

polymers. The electropolishing process smoothened the stainless steel surface significantly, without severe alteration of the surface chemistry. The silanization on the stainless steel surface provided effective reaction sites for polymer grafting. These surface modification methods resulted in stable polymer films on the stainless steel surfaces, with easy-controllability by means of a series of simple steps. In addition, the functional group introduced by polymer grafting may open the way for further modifications to be made for the purpose of drug delivery, tracer conjugation and additional polymer grafting, etc.

After performing extensive analyses of protein adsorption on various modified surfaces (smooth vs. rough, hydrophilic vs. hydrophobic), we found that the smoothened and hydrophilic surface was the best choice for reducing protein adsorption, and that a correlation truly existed between the surface properties and the protein adsorption in terms of the microroughness and hydrophilicity. Our findings may well constitute useful criteria in the field of implantable biomaterial research, such as in stent development.

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