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RESEARCH PAPER

ToF-SIMS analysis of poly(L-lysine)-*graft*-poly(2-methyl-2-oxazoline) ultrathin adlayers

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Abstract Understanding of the interfacial chemistry of ultrathin polymeric adlayers is fundamentally important in the context of establishing quantitative design rules for the fabrication of nonfouling surfaces in various applications such as biomaterials and medical devices. In this study, seven poly(L-lysine)-*graft*-poly(2-methyl-2-oxazoline) (PLL–PMOXA) copolymers with grafting density (number of PMOXA chains per lysine residue) 0.09, 0.14, 0.19, 0.33, 0.43, 0.56, and 0.77, respectively, were synthesized and characterized by means of nuclear magnetic resonance spectroscopy (NMR). The copolymers were then adsorbed on Nb_2O_5 surfaces. Optical waveguide lightmode spectroscopy method was used to monitor the surface adsorption in situ of these copolymers and provide information on adlayer masses that were then converted into PLL and PMOXA surface densities. To investigate the relationship between copolymer bulk architecture (as shown by NMR data) and surface coverage as well as surface architecture, time-of-flight secondary ion mass spectrometry (ToF-SIMS) analysis was performed. Furthermore, ToF-SIMS method combined with principal component analysis (PCA) was used to verify the protein resistant properties of PLL–PMOXA adlayers, by thorough characterization before and after adlayer exposure to human serum. ToF-SIMS analysis revealed that the chemical composition as well as the architecture of the different PLL–PMOXA adlayers indeed reflects the copolymer bulk composition. ToF-SIMS results also indicated a heterogeneous surface coverage of PLL–PMOXA adlayers with high grafting densities higher than 0.33. In the case of protein resistant surface, PCA results showed clear differences between protein resistant and nonprotein-resistant surfaces. Therefore, ToF-SIMS results combined with PCA confirmed that the PLL–PMOXA adlayer with brush architecture resists protein adsorption. However, low increases of some amino acid signals in ToF-SIMS spectra were detected after the adlayer has been exposed to human serum.

Keywords PLL–PMOXA · Graft copolymer · ToF-SIMS · PCA · Adlayer · Protein resistant

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Introduction

There is a need to produce surface coatings that create a nonfouling interface with its adjacent environment in a wide range of applications such as food processing,

packaging, heat exchangers [1], marine biofouling [2], and surgical instruments and biomedical devices [3]. In recent years, poly(oxazoline)s have attracted attention as nonfouling polymers for biomaterial and biomedical applications [4]. Earlier studies have revealed the biocompatibility of poly(oxazoline)-based system in numerous biological applications, such as stealth liposomes for cell targeting and drug delivery [4–9].

We have recently investigated surface immobilized poly(2-methyl-2-oxazoline) (PMOXA) in terms of its nonfouling properties and stability against physiological conditions, and compared it to poly(ethylene glycol) (PEG), the "gold standard" in the area [10]. Briefly, a comb–graft–copolymer system utilizing highly charged poly(L-lysine) (PLL) as a surface-anchor backbone (PLL–PMOXA, illustrated in Fig. 1) was fabricated [11, 12]. Load-bearing implant materials are often made from metals covered by their stable oxide films, such as TiO_2, Nb_2O_5, and ZrO_2. These oxide layers interact strongly with proteins at physiological conditions. Nb_2O_5 was used as a model substrate in this study due to its low isoelectric point (2.5 [13] to 4 [14]) and its high negative surface charge density (50 μC/cm^2 at pH=7.4) in aqueous solution [13]. On this surface copolymer adsorption can be performed via electrostatic interaction between the positively charged amine groups of PLL and the negatively charged substrate. The adsorption kinetics, nonfouling properties with respect to both protein [12] and bacteria [15] resistances, as well as stability [16] of the modified surfaces were investigated. The results showed that PMOXA monolayers in a brush conformation are highly nonfouling. Furthermore and important for long-term applications, this polymer showed significantly higher chemical stability compared to PEG upon exposure to physiological conditions with the latter exhibiting autocatalytic oxidative degradation [16]. This suggests that PMOXA is a strong alternative to PEG.

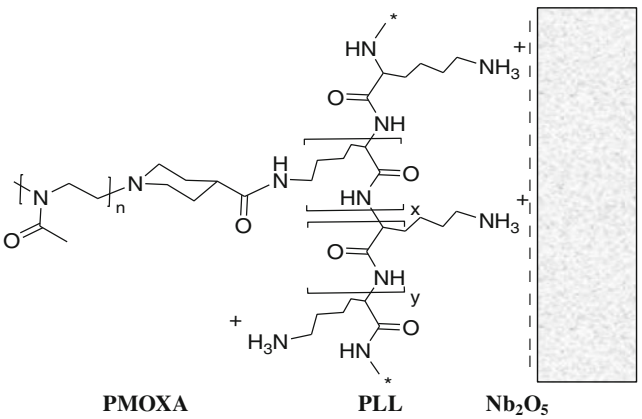


Fig. 1 Molecular structure of PLL–PMOXA. Grafting density α is defined as the number of PMOXA side chains × per number of lysine residues $(x+y)$ $(\alpha = x/(x+y))$, and **n** is the number of 2-methyl-2-oxazoline (MOXA) repeating units in the PMOXA chain. The free amines which are not coupled to PMOXA chains are positively charged at neutral pH (7.4). Thus, they serve as attachment sites with negatively charged Nb_2O_5 substrate

The quantitative evaluation of the adsorbed PLL–PMOXA monolayer by combined nuclear magnetic resonance (NMR) spectroscopy and optical waveguide lightmode spectroscopy (OWLS) techniques implied that different grafting densities of the copolymers affect the adsorption behavior and thus the physicochemical characteristics of the resulting adlayers [11], as well as a consistent picture on the interplay between PLL–PMOXA polymer architecture, kinetics of adsorption, and properties of the assembled adlayers assuming that the measured properties such as adlayer thickness or polymer mass are exclusively due to the designed polymers.

Static time-of-flight secondary ion mass spectrometry (ToF-SIMS) has been shown as ideal technique for the characterization of polymeric surfaces due to its ultrahigh surface sensitivity (sampling depth of 1–2 nm) and molecular specificity thanks to its high mass resolution which enables separation of different species with very close molecular mass values [17, 18]. It is a powerful technique for the chemical characterization of small molecules tethered to surfaces and for the differentiation of surfaces of similar chemical compositions [18]. Despite its powerful analytical capability, the large number of peaks obtained in each mass spectrum requires spectral analysis protocol, such as principal component analysis (PCA) [19]. ToF-SIMS and PCA have been used for a detailed semiquantitative investigation of the surface structure of PLL–PEG copolymer adlayers on Nb_2O_5 substrates [17]. Here, we present an evaluation of a similar graft copolymer system: PLL–PMOXA. What makes this study interesting is that different from PEG, PMOXA presents a similar chemical structure as PLL and other types of proteins, in the sense that PMOXA has a pseudopeptide structure isomeric to poly(homoalanine). We have recently reported a study where ToF-SIMS was used to characterize the homogeneity of Nb_2O_5 surfaces modified with three different PLL–PMOXA copolymers [15]. In this study, we present the results of a systematic study based on a full polymer library and use PCA to provide a quantitative insight into the relationship between bulk polymer architecture and polymer surface coverage and architecture.

A second crucial objective of this study is to get information on surface chemical changes after exposing the polymer-modified surfaces to protein solutions. Minor amounts of proteins are likely to adsorb on the surface of a grafted polymer even if the polymer chains are in brush conformation. Typical label-free techniques such as OWLS or surface plasmon resonance (SPR) have detection limits of approximately 1 ng/cm^2 (about 0.5–1 % of a saturation monolayer of typical proteins) [20] and can therefore not detect such a low but still biologically relevant surface coverage, however ToF-SIMS has much lower detection limit (parts per million to parts per

billion). Therefore, we thoroughly characterized the protein resistant PLL–PMOXA adlayer before and after exposure to full human serum.

Characteristic fragments originated from PLL and PMOXA, respectively, could be distinguished and semiquantitatively shown to be in a good agreement with combined NMR and OWLS results. Moreover, based on PCA analysis, protein-resistant PLL–PMOXA adlayers could be distinguished from nonprotein-resistant surfaces (PLL and bare Nb_2O_5 surfaces).

Experimental

Materials Initiator methyl trifluoromethylsulfonate, monomer 2-methyl-2-oxazoline, and terminating agent ethyl piperidine-4-carboxylate were purchased from Sigma-Aldrich. Acetonitrile (HPLC grade) was purchased from Fluka Chemicals. Catalysts *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride and *N*-hydroxysulfosuccinimide sodium salt (sulfo-NHS) were purchased from Sigma-Aldrich and Pierce. Buffering agent 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) was purchased from Fluka Chemicals. PLL-HBr (20 kDa) was purchased from Sigma-Aldrich.

Polymer synthesis and characterization PMOXA polymer (4 kDa) and PLL–PMOXA graft copolymers were synthesized and characterized following a previously published protocol [10, 15]. Characterization techniques involved NMR spectroscopy and matrix-assisted laser desorption/ionization-time of flight.

Substrates Silicon wafers for ToF-SIMS experiments were purchased from Si-Mat Silicon Materials Landsberg, Germany. The silicon wafers were sputter-coated with a 21-nm-thick Nb_2O_5 layer (reactive magnetron sputtering, Paul Scherrer Institute, Villigen, Switzerland). Niobia was used in view of its high negative surface charge known to result in stable immobilization of polycationic (co)polymers [13]. Waveguides (OW2400) for OWLS experiments were purchased from Microvacuum Ltd., Budapest, Hungary. Each waveguide consists of a 1-mm-thick AF45 glass substrate and a 200-nm-thick $Si_{0.75}Ti_{0.25}O_2$ waveguiding layer on the surface. An additional 6-nm-thick Nb_2O_5 layer was sputter-coated on the top of the waveguiding layer.

Substrate preparation and modification Graft copolymer adlayers were prepared by dip and rinse approach as previously described [21]. Briefly, PLL–PMOXA copolymers were dissolved at 0.1 mg/ml concentration in a filtered (0.22 μm) HEPES buffer solution containing 10 mM HEPES supplemented with 150 mM NaCl and adjusted to pH 7.4 (HEPES2). Prior to the copolymer adsorption, Nb_2O_5 substrates were ultrasonicated for 2×10 min in toluene (Fluka) followed by 2×10 min ultrasonication in 2-propanol (Fluka) and blow-drying under a stream of nitrogen. Nb_2O_5 substrates were subsequently cleaned by oxygen plasma treatment for 2 min (plasma cleaner/sterilizer PDC-32G, Harrick Scientific Products Inc.). A 50 μl copolymer solution were then placed onto the precleaned substrates completely covering their surfaces. Copolymer adsorption was allowed to proceed for around 2 h, followed by extensive washing with HEPES2 solution, ultrapure water, and finally blow-drying under a stream of nitrogen. The immobilized PLL–PMOXA polymer on Nb_2O_5 surface is illustrated in Fig. 1.

For investigation on protein resistance, polymer-modified surfaces were prepared as described above. Prior to incubation with human serum, the polymer layer was hydrated for at least 30 min by incubating the surface in HEPES2. A 50 μl human serum (control serum, Precinorm® U, LOT: 171 074-01, reconstituted in ultrapure water) was then placed onto the rehydrated polymeric interface. After 15 min of exposure to human serum, the samples were thoroughly rinsed with ultrapure water and dried under a stream of nitrogen.

Quantitative and in situ monitoring of PLL–PMOXA adsorption by OWLS OWLS 110 with BioSense 2.2 software, Microvacuum Ltd., Budapest, Hungary was used to analyze the adsorbed mass of copolymers on Nb_2O_5 surfaces ("dry mass" per unit area). The OWLS experiments to each copolymer were performed following a procedure as previously described [11, 12].

ToF-SIMS analysis Positive and negative ToF-SIMS spectra were acquired in the mass range of 0–1,000 *m/z* using the ION-TOF TOF-SIMS V instrument (Germany), equipped with a reflectron ToF analyzer. Secondary ions were obtained following surface bombardment using Bi_3^+ primary ions (0.3 pA pulsed ion current) on a 200×200 μm^2 surface area, with a total ion dose of ~5×10^{11} ions/cm^2. A mass resolution ($m/\Delta m$) of ~7,000 was typically achieved at nominal *m/z* 41 ($C_3H_5^+$) for positive spectra and at nominal *m/z* 36 (C_3^-) for negative spectra, respectively. A minimum of two samples with at least three different areas per sample were analyzed for each sample type.

Evaluation on fragment peaks and their intensities from the acquired ToF-SIMS spectra was performed on IonSpec, ToF-SIMS software V4.1. Each positive spectrum was calibrated using $[C]^+$, $[C_2H_2]^+$, $[C_3H_4]^+$, $[C_4H_3]^+$, $[Nb]^+$, and $[NbH]^+$ peaks. Each negative spectrum was calibrated using $[C]^-$, $[CH_2]^-$, $[F]^-$, $[CHO]^-$, $[CH_3O]^-$, and $[NbO_2]^-$ peaks. Furthermore, the peak intensities in ToF-SIMS spectra are dependent on the experimental conditions. Therefore, each peak was normalized to the total sum of peak intensities of the corresponding spectrum.

Determination of characteristic fragments After calibration of spectra, characteristic fragments from each element on the surface were determined, by comparing three different surfaces: bare Nb_2O_5, PLL adlayer on Nb_2O_5, and PLL–PMOXA adlayer on Nb_2O_5 (see Electronic supplementary material (ESM) Fig. S1).

Multivariate analysis After calibration of spectra, as many significant mass peaks as possible (>200 peaks) were added to the peak list. Prominent peaks in the range of mass over charge ratio m/z = 1–200 amu were selected, including published amino acid fragments originated from proteins [21, 22], unambiguously assigned by means of comparison between experimental and theoretical masses of the secondary ions (mass deviation ≤100 ppm), and used for multivariate data analysis. Peaks that are originated from contaminants such as Na^+ and K^+ in positive secondary ion mass spectra were excluded to avoid distinction of surfaces due to different level of contaminations. Before multivariate analysis, each peak was normalized to the total of intensities of the corresponding spectrum. Furthermore, mean-centered principal components are used to insure that the differences between samples were due to differences in the sample variances instead of differences in sample means. The multivariate analysis was performed in MATLAB R2009a. The program code for multivariate analysis was developed according to the PCA principles [19] and available in ESM.

Results and discussion

Surface density of PMOXA and PLL

We have synthesized seven PLL–PMOXA graft copolymers with PMOXA molecular weight of 4 kDa. Based on NMR characterization, the grafting densities (α) of the copolymers were 0.09, 0.14, 0.19, 0.33, 0.43, 0.56, and 0.77. OWLS measurements provided information on the PLL–PMOXA adlayer mass (nanogram per square centimeter), which could then be converted into PMOXA surface density (n_{PMOXA}) and PLL surface density (n_{PLL}) [11]. Figure 2a, b show the surface density n_{PMOXA} and n_{PLL}, respectively, for the PLL–PMOXA adlayer as a function of α.

As seen in Fig. 2a, n_{PMOXA} increased linearly with increasing α, from low to medium values of 0.33. At higher grafting density, a decrease in the n_{PMOXA} was observed. The explanation of this phenomenon has been published [11, 12]. As α increased, the concentration of positive charges originating from the ammonium groups on the PLL backbone decreased, while steric hindrance of the copolymer structure increased. These two factors reduced the thermodynamic driving force for the copolymer adsorption process. Accordingly, Fig. 2b demonstrates a decrease in n_{PLL} for all PLL–PMOXA copolymers as the grafting density increases.

ToF-SIMS characterization of PLL–PMOXA adlayers

Typical positive secondary ion mass spectra of bare Nb_2O_5, PLL adlayer, and PLL–PMOXA (α = 0.33, gives the highest surface density of PMOXA) adlayer can be seen in ESM Fig. S2. Prominent peaks in the mass spectra of the adlayers were found to be associated with the chemical structure of PLL and PLL–PMOXA. Significant differences between the spectra were observed when high mass resolution spectra were displayed. The high mass resolution in ToF-SIMS ($m/\Delta m \approx 7{,}000$) allowed discrimination between chemical species with very close m/z values and thereby enabled the elucidation of chemical information that identified fragment ions diagnostic of particular adlayers.

To gain more understanding on the interfacial chemistry of the adlayers, quantitative data evaluation was performed.

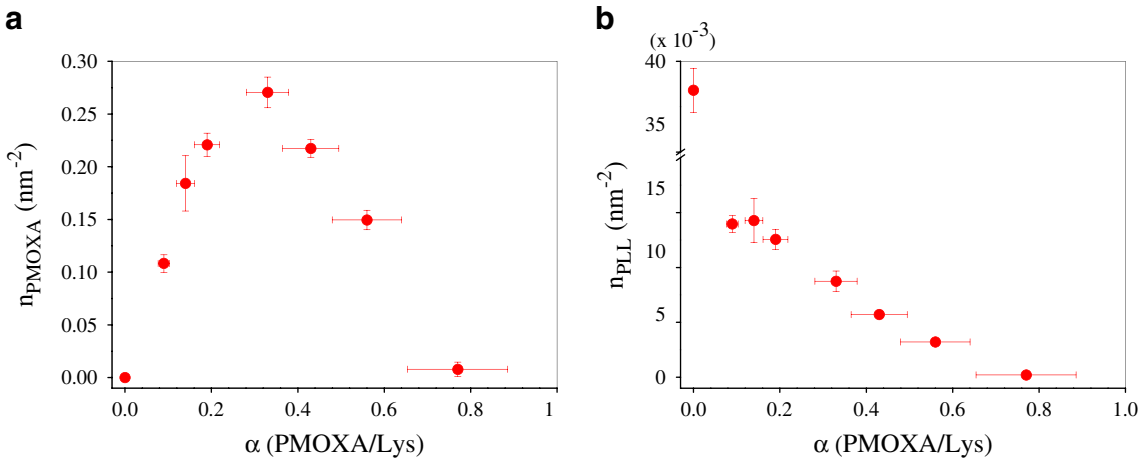


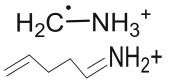
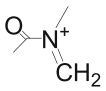
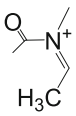
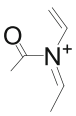
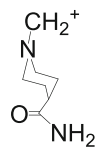
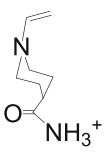
Fig. 2 Surface density of **a** PMOXA and **b** PLL as a function of grafting density α

Only fragments that originate *exclusively* from a specific component of the interfacial chemical structure of the adlayers were selected and these fragments are summarized in Table 1. These fragments were at m/z values of 31 (CH_5N^+) and 84 ($C_5H_{10}N^+$) that were assigned to PLL, 86 ($C_4H_8NO^+$), 100 ($C_5H_{10}NO^+$), 112 ($C_6H_{10}NO^+$), 141 ($C_7H_{13}N_2O^+$), and 155 ($C_8H_{15}N_2O^+$) to PMOXA and 93 (Nb^+), 109 (NbO^+), and 125 (NbO_2^+) to Nb_2O_5.

Figure 3 shows the total intensity of the characteristic peaks assigned to PMOXA (Fig. 3a), PLL (Fig. 3b), and Nb_2O_5 (Fig. 3c), respectively, as a function of α. The results in Fig. 3a, b are nicely correlated with the results obtained by OWLS (shown in Fig. 2). The sum of PMOXA-characteristic fragment intensities (I_{PMOXA}^+) increased linearly as α increased from 0 to 0.33, which then decreased linearly to an α of 0.77 (Fig. 3a). However, PLL-characteristic fragment intensities (I_{PLL}^+) decreased exponentially (Fig. 3b).

Figure 3c shows a complementary picture of Fig. 3a. Given the sampling depth of ToF-SIMS (i.e., 1–2 nm), the total intensity of Nb_2O_5-characteristic fragments (I_{Nb2O5}^+) from the underlying substrate is affected by the thickness and uniformity of the PLL–PMOXA overlayer. As α increases from 0 to 0.33, the PMOXA surface density increased, and the Nb_2O_5 substrate was "shielded" by the dense PMOXA chains. This resulted in a decrease in the I_{Nb2O5}^+ from α of 0 to 0.33. However, at α above 0.33, the I_{Nb2O5}^+ increases despite the increase of grafting density of the PLL–PMOXA copolymers. To explain this phenomenon, here we propose three models of PLL–PMOXA adlayer configuration. The first model (Fig. 4a) illustrates a homogeneous brush layer, formed by

Table 1 A summary of characteristic positive secondary ion fragments and their proposed chemical structures

Nominal mass [*m/z*]	Characteristic positive secondary ion fragments	Proposed structure
	Nb_2O_5:	-
93	Nb^+	
109	NbO^+	
125	NbO_2^+	
	PLL:	
31	CH_5N^+	
84	$C_5H_{10}N^+$	
	PMOXA:	
86	$C_4H_8NO^+$	
100	$C_5H_{10}NO^+$	
112	$C_6H_{10}NO^+$	
141	$C_7H_{13}N_2O^+$	
155	$C_8H_{15}N_2O^+$	

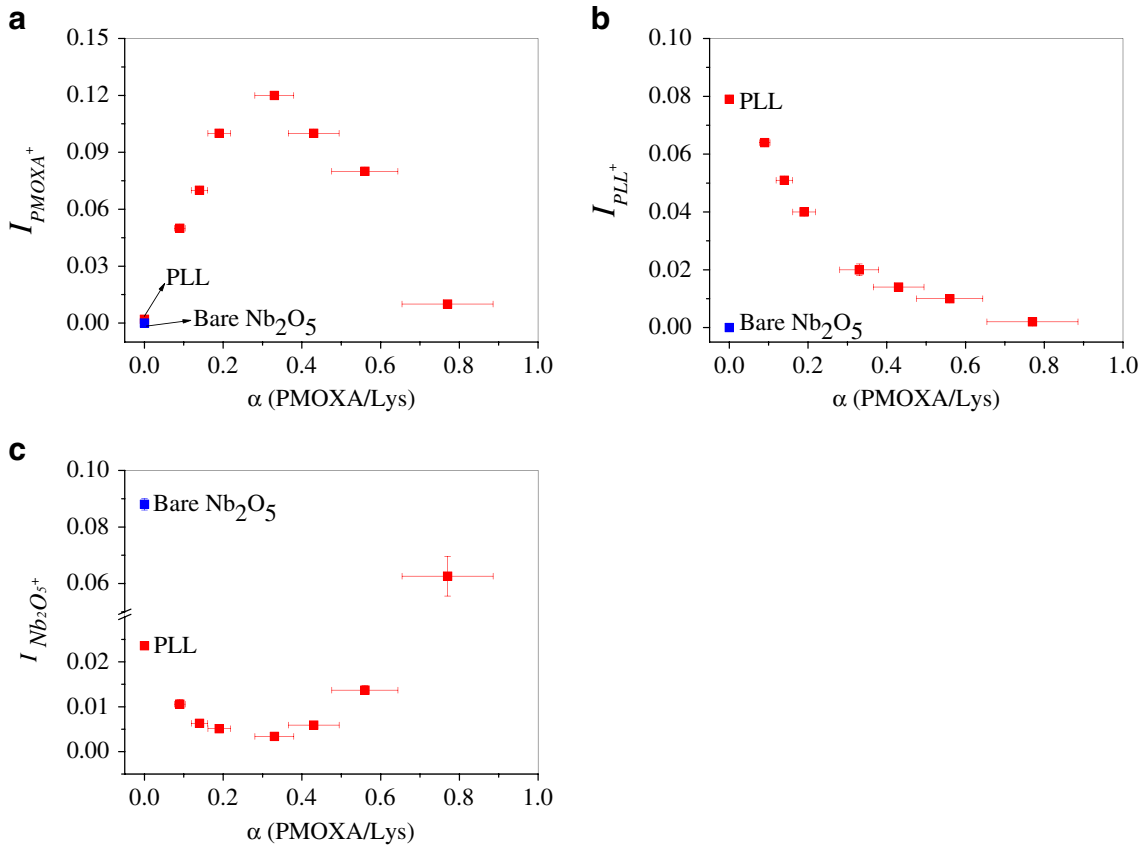


Fig. 3 ToF-SIMS analysis of the different PLL–PMOXA copolymer adlayers, PLL, and bare Nb_2O_5 surface: the total intensity of positive secondary ion intensities (normalized to the total ion intensities of the corresponding spectra) as a function of grafting density α for **a** PMOXA-, **b** PLL-, and **c** Nb_2O_5-characteristic fragments calculated for the different samples. *Vertical error bars* correspond to standard deviations of data obtained from two independent samples with three different measurement areas per sample

PLL–PMOXA copolymer with optimum grafting density, in this case 0.33. As α increases above 0.33, the main backbone becomes stiffer and less flexible, leading to two possible configurations illustrated in Fig. 4b, c. In Fig. 4b, the surface is relatively homogeneous with a thinner PMOXA layer compared to the brush PLL–PMOXA ($\alpha = 0.33$) adlayer. However, in Fig. 4c, the surface is heterogeneous with local regions exhibiting high PMOXA density and regions exposing bare Nb_2O_5 substrate.

In order to suggest the most possible adlayer configuration of PLL–PMOXA with $\alpha > 0.33$, we use two parameters: PMOXA enrichment (PE) and overlayer thickness (OT) that could be derived from PMOXA, PLL, and Nb_2O_5 characteristic fragment intensities (Eqs. 1 and 2) [17]:

$$\mathrm{PE} = \frac{I_{\mathrm{PMOXA}}}{I_{\mathrm{PLL}} + I_{\mathrm{Nb_2O_5}}} \quad (1)$$

$$\mathrm{OT} = \frac{I_{\mathrm{PLL}} + I_{\mathrm{PMOXA}}}{I_{\mathrm{Nb_2O_5}}} \quad (2)$$

PE values are plotted as a function of OT in Fig. 5a. As α increases form 0 (pure PLL) to 0.33, both OT and PE increase. Interestingly above 0.33, the OT decreases as α increases, while the PE values remains relatively high. For PLL–PMOXA with $\alpha = 0.43$, the PE value is similar to that with $\alpha = 0.33$, although the OT value was significantly lower. This phenomenon indicates that the surface became heterogeneous as α increases above 0.33 (as proposed and illustrated in Fig. 4c). In the homogeneous case (as illustrated in Fig. 4b), we would expect that high intensity of PMOXA-related fragments (high PE value) is concomitant with low intensity of Nb_2O_5-related fragments (which should result in a high OT value) due to the "shielding effect" of the copolymer adlayer and the low sampling depth of ToF-SIMS technique. The heterogeneous case is further evidenced in the case of PLL–PMOXA with $\alpha = 0.56$. Although the PE value was significantly higher than the adlayers formed from PLL–PMOXA with $\alpha = 0.09$, 0.14, and 0.19, the OT value was significantly low.

Figure 5b is complementary to Fig. 5a, with the sum of Nb_2O_5 signal intensities (I_{Nb2O5^+}) plotted as a function of n_{PMOXA}. Figure 5b shows that there is an outlier in the I_{Nb2O5^+} vs. n_{PMOXA} trendline, where the I_{Nb2O5^+} shows a higher value than the trendline. The outlier corresponds to

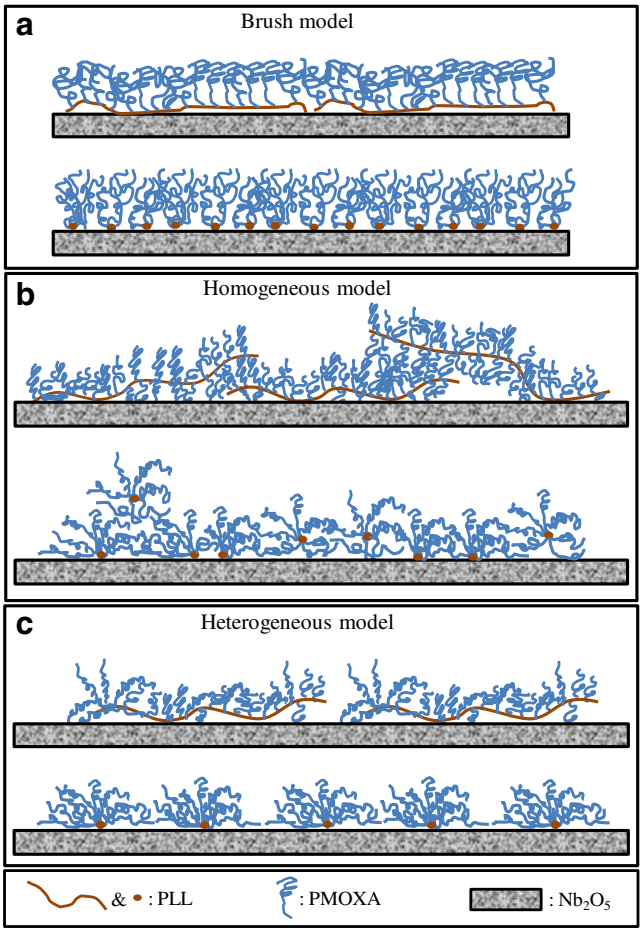


Fig. 4 Proposed adlayer configurations: **a** homogeneous model with brush configuration of polymer chains, resulting in a dense adlayer, **b** homogeneous model with some overlaps of polymer chains and thinner adlayer, and **c** heterogeneous model with lateral variation of the surface. For each figure, the polymer structure is graphically represented both in the direction of the PLL backbone and perpendicular to it

n_{PMOXA} value of 0.17, that is formed by PLL–PMOXA α=0.56 copolymer. This information supports the suggested heterogeneous model as proposed in Fig. 4c, presenting an adlayer with lateral variation of very dense PMOXA chains (high n_{PMOXA}) and exposed bare Nb_2O_5 surface (high I_{Nb2O5}^{+}).

Contrary to positive secondary ion spectra, it was observed that no fragment peaks were exclusively characteristic to PLL from the negative secondary ion mass spectra. However, characteristic fragments for PMOXA and Nb_2O_5 were identified in the negative secondary ion mass spectra and the results are summarized in ESM Table S1 and Fig. S3.

Verification of protein resistance

In this study, we investigated the protein resistance capability of all PLL–PMOXA adlayers using a previously published protocol [11]. We found that the best protein resistance was achieved by surfaces modified with PLL–PMOXA (α=0.33), with adsorbed protein mass of <1 ng/cm^2 (OWLS spectrum not shown). In order to confirm that the insignificant mass increase was not due to replacement of polymer molecules by the protein molecules, ToF-SIMS and multivariate analysis was performed on PLL–PMOXA (α=0.33) adlayer presenting "optimum" brush [15] conformation. Bare Nb_2O_5 and PLL adlayer on Nb_2O_5, which are not resistant to protein adsorption, served as controls.

Figure 6a, b show scores and loadings from principal component 1 (PC1) that captured 61 % of the variance. In Fig. 6a, the PLL–PMOXA (α=0.33) adlayer exhibited negative scores, indicating a dense PMOXA layer on the surface. Figure 6b depicts that the PC1 separated PMOXA-related peaks (loaded negatively) from any other peaks. After exposure to human serum, the PLL–PMOXA (α=0.33) adlayer still exhibited a negative score, indicating that human serum proteins did not significantly adsorb to the surface. This result

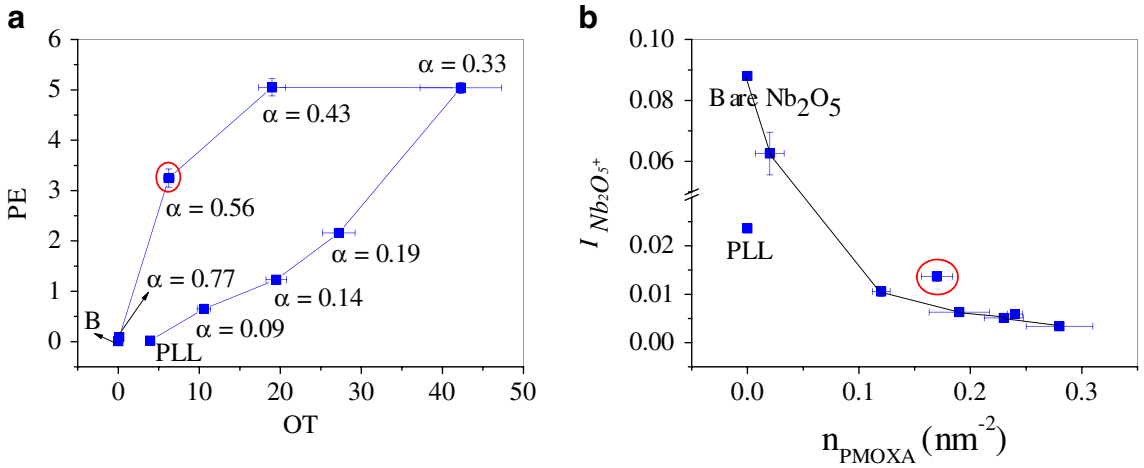


Fig. 5 **a** PMOXA enrichment (PE) as a function of overlayer thickness (OT) and **b** the sum of Nb_2O_5 characteristic positive secondary ion signal intensities (I_{Nb2O5}^{+}) as a function of PMOXA surface density (n_{PMOXA})

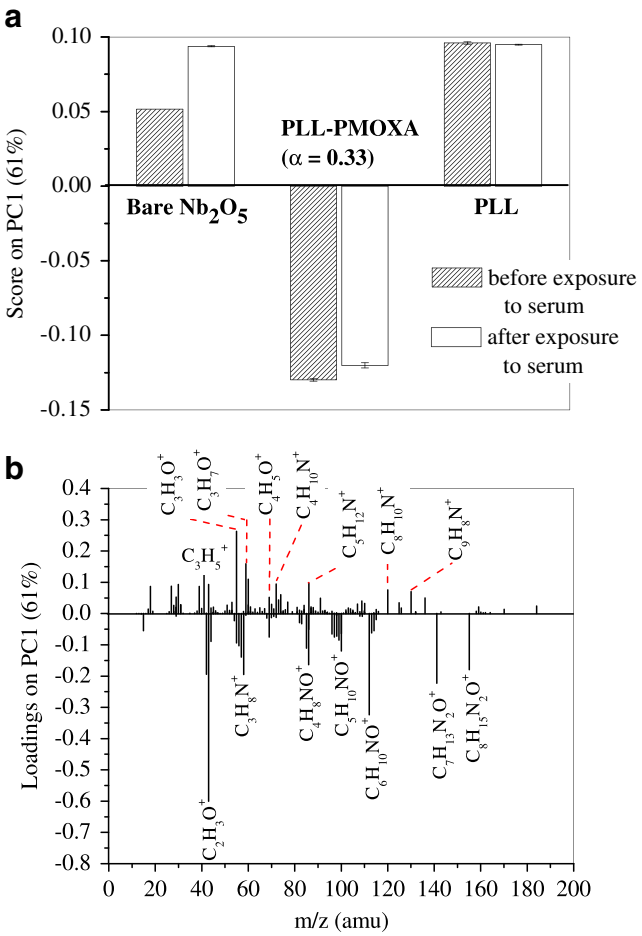


Fig. 6 **a** Scores and **b** loadings on principal component 1 (PC1) obtained from PCA applied to positive ToF-SIMS spectra of bare Nb_2O_5, PLL–PMOXA (α=0.33), and PLL adlayers on Nb_2O_5 substrates, as well as after exposure of the surfaces to human serum for 15 min. PC1 captured 61 % of the variance in the evaluated spectra, and could distinguish PLL–PMOXA (α=0.33) adlayers (both before and after exposure to human serum) from any other surfaces

confirmed the protein resistance of PLL–PMOXA brush surfaces as suggested by OWLS analysis. In contrast, bare and PLL-coated Nb_2O_5 surfaces after incubation in human serum exhibited positive scores since the substrate- and amino acid-related peaks loaded positively (Fig. 6b).

Although statistically not significant compared to those on bare Nb_2O_5 and PLL adlayer on Nb_2O_5, several amino acid signals showed a small increase for PLL–PMOXA (α=0.33) adlayer after exposure to human serum. Table 2 shows the ratios of amino acid signal intensities before and after exposure to human serum for the different surfaces.

Serum is derived from blood plasma, with clotting factors removed [23]. One of the serum major constituents is albumin, which is known to bind and transport small molecules and peptides within the human circulatory system [24]. Human serum is thus a complex body fluid that contains 60–80 mg/ml of various proteins in addition to various small molecules such as salts, lipids, amino acids, and sugars [23].

Our finding confirmed that coating a bare Nb_2O_5 surface with PLL–PMOXA (α=0.33) brush layer significantly reduced protein adsorption. However, there is an indication that a minor amount of proteins stick on the adlayer. Although the reason of this phenomenon is not clear to us at this stage, we speculate that the increase of some amino acid signals (most of them belong to hydrophobic amino acids) on the protein-resistant PLL–PMOXA adlayer might be due to diffusion of low-molecular-weight proteins to the PLL/Nb_2O_5 interface and interaction between the amino acids with PLL through hydrophobic interaction. Furthermore, this finding also confirmed the sensitivity of ToF-SIMS technique to detect very low surface coverage that could not be done using optical surface characterization techniques such as OWLS and SPR.

Table 2 Amino acid fragments [21, 22] showing increased signal intensities before and after exposure to human serum proteins for PLL–PMOXA (α=0.33) adlayer

Nominal mass [m/z]	Characteristic positive secondary ion fragments	Assigned to amino acid	Ratio of signal intensity before and after exposure to human serum		
			Bare Nb_2O_5	PLL adlayer	PLL–PMOXA (α=0.33) adlayer
34.9974	H_3S^+	Cysteine	18.7	61.8	1.5
59.0503	$CH_5N_3^+$	Arginine	9.6	29.7	3.2
60.0462	$C_2H_6NO^+$	Serine	53.9	55.7	1.7
60.0586	$C_3H_8O^{*+}$	Threonine	124.9	207.0	3.7
61.0122	$C_2H_5S^+$	Methionine	10.5	45.3	2.7
61.0545	$C_2H_7NO^{*+}$	Alanine	31.4	41.9	1.9
102.0584	$C_4H_8NO_2^+$	Glutamic acid	19.1	21.8	1.7
103.0578	$C_5H_{11}S^+$	Methionine	6.2	10.9	1.6
104.1131	$C_5H_{14}NO^+$	Leucine	73.0	114.4	2.6
120.0975	$C_8H_{10}N^+$	Phenylalanine	91.7	38.6	2.5
130.0762	$C_9H_8N^+$	Tryptophan	41.7	60.0	2.1

Conclusion

Despite the similar chemical structure between PLL and PMOXA, characteristic fragments for the former and the latter could be distinguished in positive ToF-SIMS spectra. ToF-SIMS results in conjunction with those from OWLS provide evidence that the PLL–PMOXA adlayer indeed reflect the composition of the corresponding bulk polymers as determined quantitatively using NMR spectroscopy. Similar to PLL-PEG system, TOF-SIMS spectra of PLL–PMOXA adlayer were principally affected by the density of the PMOXA chains in the adlayer, which corresponds to grafting density (PMOXA/Lys ratio) of the copolymers.

Principal component analysis of positive ToF-SIMS spectra on adlayers originated from PLL–PMOXA of grafting density 0.33 before and after exposure to human serum confirmed the protein-resistant properties of PMOXA brush, as previously indicated by OWLS data. However, ToF-SIMS data indicate a low coverage of proteins on the protein resistant PLL–PMOXA adlayer. We speculate that this is due to diffusion of low-molecular-weight proteins through the PMOXA brush layer to the PLL/Nb_2O_5 interface, and hydrophobic interaction with PLL main backbone of the PLL–PMOXA draft copolymer.

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References

1. Bansal B, Chen XD (2006) A critical review of milk fouling in heat exchangers. Comp Rev Food Sci Food Saf 5(2):27–33
2. Railkin AI (2004) Marine biofouling colonization processes and defenses. CRC Press, Boca Raton
3. Campoccia D, Montanaro L, Arciola CR (2006) The significance of infection related to orthopedic devices and issues of antibiotic resistance. Biomaterials 27(11):2331–2339
4. Hoogenboom R (2009) Poly(2-oxazoline)s: a polymer class with numerous potential applications. Angew Chem Int Ed 48(43): 7978–7994
5. Woodle MC, Engbers CM, Zalipsky S (1994) New amphipatic polymer–lipid conjugates forming long-circulating reticuloendothelial system-evading liposomes. Bioconjug Chem 5(6):493–496
6. Zalipsky S, Hansen CB, Oaks JM, Allen TM (1996) Evaluation of blood clearance rates and biodistribution of poly(2-oxazoline)-grafted liposomes. J Pharm Sci 85(2):133–137
7. Cheon Lee S, Kim C, Chan Kwon I, Chung H, Young Jeong S (2003) Polymeric micelles of poly(2-ethyl-2-oxazoline)-block-poly(ε-caprolactone) copolymer as a carrier for paclitaxel. J Control Release 89(3):437–446
8. Broz P, Benito SM, Saw C, Burger P, Heider H, Pfisterer M, Marsch S, Meier W, Hunziker P (2005) Cell targeting by a generic receptor-targeted polymer nanocontainer platform. J Control Release 102(2): 475–488
9. Ranquin A, Versees W, Meier W, Steyaert J, Van Gelder P (2005) Therapeutic nanoreactors: combining chemistry and biology in a novel triblock copolymer drug delivery system. Nano Lett 5(11): 2220–2224
10. von Erlach T, Zwicker S, Pidhatika B, Konradi R, Textor M, Hall H, Luhmann T (2011) Formation and characterization of DNA–polymer-condensates based on poly(2-methyl-2-oxazoline) grafted poly(L-lysine) for non-viral delivery of therapeutic DNA. Biomaterials 32:5291–5303
11. Pidhatika B, Möller J, Vogel V, Konradi R (2008) Nonfouling surface coatings based on poly(2-methyl-2-oxazoline). CHIMIA Int J Chem 62:264–269
12. Konradi R, Pidhatika B, Mühlebach A, Textor M (2008) Poly-2-methyl-2-oxazoline: a peptide-like polymer for protein-repellent surfaces. Langmuir 24(3):613–616
13. Kenausis GL, Voros J, Elbert DL, Huang N, Hofer R, Ruiz-Taylor L, Textor M, Hubbell JA, Spencer ND (2000) Poly(L-lysine)-G-poly(-ethylene glycol) layers on metal oxide surfaces: attachment mechanism and effects of polymer architecture on resistance to protein adsorption. J Phys Chem B 104(14):3298–3309
14. Kosmulski M (2001) Chemical properties of material surfaces. In: Hubbard AT (ed) Surfactant science series, vol 102. Marcel Dekker, New York, p 129
15. Pidhatika B, Möller J, Benetti EM, Konradi R, Rakhmatuliina E, Muehlebach A, Zimmermann R, Werner C, Vogel V, Textor M (2010) The role of the interplay between polymer architecture and bacterial surface properties on the microbial adhesion to polyoxazoline-based ultrathin films. Biomaterials 31:9462–9472
16. Pidhatika B, Rodenstein M, Chen Y, Rakhmatullina E, Mühlebach A, Acikgöz C, Textor M, Konradi R (2012) Comparative stability studies of poly(2-methyl-2-oxazoline) and poly(ethylene glycol) brush coating. Biointerphases 7(1):1–15
17. Wagner MS, Pasche S, Castner DG, Textor M (2004) Characterization of poly(L-lysine)-graft-poly(ethylene glycol) assembled monolayers on niobium pentoxide substrates using time-of-flight secondary ion mass spectrometry and multivariate analysis. Anal Chem 76(5):1483–1492
18. Al-Bataineh SA, Jasieniak M, Britcher LG, Griesser HJ (2008) TOF-SIMS and principal component analysis characterization of the multilayer surface grafting of small molecules: antibacterial furanones. Anal Chem 80(2):430–436
19. Hardle W, Simar L (2007) Applied multivariate statistical analysis. Springer, Berlin
20. Vörös J, Ramsden JJ, Csúcs G, Szendro I, De Paul SM, Textor M, Spencer ND (2002) Optical grating coupler biosensors. Biomaterials 23(17):3699–3710
21. Pasche S, DePaul SM, Voros J, Spencer ND, Textor M (2003) Poly(L-lysine)-graft-poly(ethylene glycol) assembled monolayers on niobium oxide surfaces: a quantitative study of the influence of polymer interfacial architecture on resistance to protein adsorption by ToF-SIMS and in-situ OWLS. Langmuir 19(22):9216–9225
22. Michel R, Pasche S, Textor M, Castner DG (2005) Influence of PEG architecture on protein adsorption and conformation. Langmuir 21(26):12327–12332
23. Adkins JN, Varnum SM, Auberry KJ, Moore RJ, Angell NH, Smith RD, Springer DL, Pounds JG (2002) Toward a human blood serum proteome: analysis by multidimensional separation coupled with mass spectrometry. Mol Cell Proteomics 1(12):947–955
24. Tirumalai RS, Chan KC, Prieto DA, Issaq HJ, Conrads TP, Veenstra TD (2003) Characterization of the low molecular weight human serum proteome. Mol Cell Proteomics 2(10):1096–10103