Deposition and Bioadhesion Properties of Polymer Multilayers: An *in-situ*-ATR-FTIR-Study

Martin Müller*[1], Theresia Rieser[1], Marcello Köthe[1], Bernd Keßler, Marcela Brissova[2] and Klaus Lunkwitz[1]

[1]Institute for Polymer Research Dresden e.V., D-01069 Dresden, Germany
[2]Vanderbilt University, Department of Medicine, Nashville, TN 37232, USA

SUMMARY: The multilayer formation by consecutive deposition of oppositely charged polyelectrolytes and the protein adsorption at the outermost layer was investigated by *in-situ* attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy. For the polyelectrolyte multilayer generation the polycation / polyanion pairs poly(diallyldimethylammonium chloride) (PDADMAC) / sodium poly(maleic acid-co-propylene) (PMAPP) and poly(ethyleneimine) (PEI) / poly(acrylic acid) (PAC) respectively, were used. The ν(CO)- and the $\nu_{as}(COO^-)$-band due to COOH- und COO^--groups of the polyacid/polyanion as well as the ν(OH) band due to desorbed water were used for monitoring the multilayer build up.

By the multilayer surface modification we were able to diminuish or enhance significantly the adsorption of the model plasma protein human serum albumine (HSA), as a measure for bioadhesion, in dependence on the character of the outermost layer. This protein resistence mainly was attributed to the repulsion between the like charges of the outermost adsorbed polyanion layer (e.g. PAC) und the acidic HSA (IEP=4.7). Vice versa it could be shown, that HSA was bound by an outermost polycation layer by a factor >10 stronger compared to an outermost polyanion layer. Furthermore studies on protein adsorption at multilayers built up by the reactive polymer pair poly(butadienepoxide) (PBDE) / poly(allylamine) (PAA) were introduced.

INTRODUCTION

Polymer or inorganic surfaces can be modified elegantly using selected reactive polymer pairs (A/B) for the alternating build up of multilayers, if one polymer component (A) is able to both anchor at the (premodified) surface and create a new reactive surface for the binding of the other polymer (B). This methodology was initiated by Decher[1)] especially for polyelectrolytes (PEL), applying a consecutive dip/rinse protocol of the polymer components. In the past, studies of this multilayer systems were dedicated to the architecture of vertically highly ordered layer systems offering applications for non linear optical systems and to the possibility to introduce functional interlayers (C) for microelectronic devices and biosensors.

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Beneath a defined surface property (i) (functionality, charge, wetability, surface energy) the main advantages of multilayers (n > 4) over monolayer systems are

- lateral homogeneity (ii)
- low surface roughness (iii)
- fine tuning of the layer thickness (iv)
- high shear stability (v)
- easy to handle (vi)

One potential application of multilayer assemblies which was put not that much attention on refers to their susceptibility to bioadhesion/fouling processes in contact to aqueous biological fluids (blood, food). Recently, an unique concept was reported to enhancec the protein resistance of polypropylene membranes on the basis of PEL multilayers[2], which seems to be promising for the development of medical or food processing devices, container materials or tubing in contact with biological fluids.

In order to elucidate this interfacial phenomenon at the molecular level, multilayers composed of the polycation/polyanion pairs poly(diallyldimethylammonium chloride) (PDADMAC) / poly(maleic acid-co-propylene) (PMAPP) (i) and poly(ethyleneimine) (PEI) / poly(acrylic acid) (PAC) (ii) were deposited on planar model surfaces (Si-crystals). As analytical method the surface and layer sensitive *in-situ*-ATR-FTIR-Spectroscopy was chosen, whereby this method was used, to our knowledge for the first time, as *on-line* control of the multilayer deposition[3] and further for the quantitative determination of the protein adsorption at the multilayers.

EXPERIMENTAL PART

Surface

As model surfaces for the polyelectrolyte multilayer modification plasma cleaned (plasma chamber PDC-32G, Harrick (distributed by Starna, Pfungstadt), 1 Torr, 2 min, 100W) Internal Reflection Elements (IRE) of Si ($n_1 = 3.5$) were used.

Polyelectrolyte Multilayers

The polycations poly(diallyldimethylammonium chloride) (PDADMAC, linear, M_w = 160.000 g/mol, W. Jaeger, Fraunhofer Institut für Angewandte Polymerforschung),

poly(ethyleneimine) hydrochloride (PEI, Aldrich, M_W = 750.000 g/mol) and the polyanions poly(maleic acid-co-propylene) (PMAPP, Leuna, M_w = 23.000 g/mol), poly(acrylic acid) (PAC, Sigma, M_w = 90.000 g/mol) were used without further preparation and were dissolved in deionized water (Millipore, 18.2 MΩ) to a concentration of 5-10 mmol/l. The multilayers of oppositely charged polyelectrolytes were fabricated by consecutive depositing/rinsing cycles above the Si-IRE in the sample compartment of the ATR-IR sorption cell (IPF Dresden) according to the stream coating procedure described in[3]. Between every polyelectrolyte addition, the sorption cell was carefully rinsed with water.

Protein Adsorption

Human serum albumine ($M_r \approx$ 66.000) was supplied by Sigma and was dissolved in PBS buffer (Fluka, 1 mg/ml, pH = 7.4). The protein adsorption measurements were performed directly at the freshly prepared multilayers in the ATR-IR sorption cell.

ATR-FTIR-Spectroscopy

The *In-Situ-ATR-FTIR Apparatus for Sorption Measurements* (U. P. Fringeli, University of Vienna, OPTISPEC, Zürich), consisting of a special mirror setup and the *in-situ*-sorption cell (IPF Dresden) was used on a commercial rapid scan FTIR-spectrometer (IFS 28, BRUKER) equipped with globar source and MCT detector, as described elsewhere[3]. ATR-FTIR absorbance spectra were recorded by the SBSR-(Single Beam-Sample Reference)-method, whereby single channel spectra $I_{S,R}$ were recorded of both the upper (S) and lower (R) half of the Si-IRE (50*20*2 mm^3). Above the sample and reference half are two liquid chambers (S, R) sealed by O-rings (Viton), which are filled with polyelectrolyte or protein solution (S-chamber) and with water (R-chamber), respectively. Ratioing of the single channel spectra according to $A_{SBSR} = -\log (I_S/I_R)$ resulted in absorbance spectra (A_{SBSR}), which exhibited a proper compensation of the background absorptions due to the SiO_x-layer, the solvent (water), the water vapor (spectrometer) and ice on the MCT detector window.

RESULTS AND DISCUSSION

Deposition of Polyelectrolyte Multilayers

Polymer multilayers were fabricated by the consecutive alternate build up of oppositely charged polyelectrolytes, which initially was published by Decher[1]. A modified fill/rinse

technique ('stream coating') within the sorption cell was used to create multilayers on plasma treated Si IREs, which was monitored *in-situ* by ATR-IR-spectroscopy. Whereas in a recent paper we presented results on the *in-situ* monitoring of PEI/PAC multilayer deposition on both plasma treated Si-IREs and thin casted polypropylene films[3], here we show the alternate layer built up of PDADMAC and the poly(maleic acid-co-propylene) (PMAPP), which is an additional suitable carboxyl containing weak polyanion, at the Si-IRE surface.

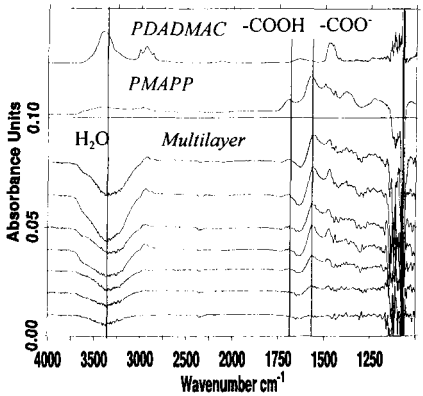

Fig. 1: Lower part (from bottom to top): *in-situ*-ATR-FTIR-spectra monitoring the alternated deposition of PDADMAC and PMAPP after the adsorption steps 1, 5, 10, 15, 20, 25 and 30, respectively, at the Si-IRE (n_1=3.5, incident angle θ= 45°, 11 active reflexions). Upper part: bulk IR spectra of PDADMAC and PMAPP.

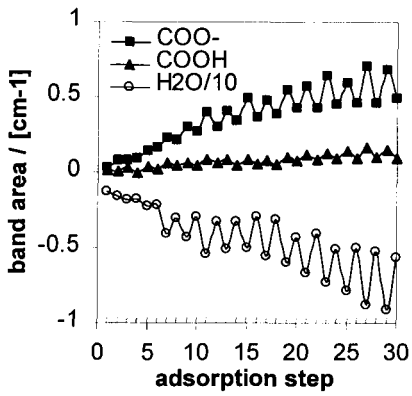

Fig. 2: Integrated areas of the ν(OH) (scaled by 1/10), ν(COO^-) and ν(C=O) bands in the ATR-FTIR spectra (Fig. 1) on the alternated PDADMAC / PMAPP multilayer deposition plotted against the adsorption step (PDADMAC: odd steps, PMAPP: even steps).

In Fig. 1 ATR-FTIR-spectra are shown, which were recorded after every adsorption step of the two polyelectrolyte components PDADMAC and PMAPP at the SiO_x surface. For the on-line monitoring of multilayer formation we used both the ν(CO)-band (COOH-groups) at 1705 cm^{-1} and the $\nu_a(COO^-)$-band (COO^--groups) at 1580 cm^{-1} of PMAPP. For comparison the IR spectra of the single components are shown on the top. An assignment of the relevant IR bands is given in Table 1. Especially, for the center wavenumber of the $\nu_a(COO^-)$ band we observed a significant deviation for the single bulk PMAPP and for PMAPP incorporated in

the multilayers, which might be attributed to the ion pair formation between the carboxylic and the ammonium groups.

Table 1. Assignment of the infrared bands of the polyelectrolyte components in PDADMAC/PMAPP multilayers

wavenumber / [cm^{-1}]	assignment	component
3700-3000	ν(OH)	water
1705 (+/-5)	ν(C=O)	PMAPP
1580 (+/-10)	$\nu_a(COO^-)$	PMAPP
1480	δ(CH	PDADMAC
1370 (+/-10)	$\nu_s(COO^-)$	PMAPP

The integrated areas of these bands are plotted against the adsorption step in Fig. 2, proving the multilayer growth by the increase of the ν_a(COO) band. However, there is no linear dependence of the band area on the number of adsorption steps, since the more single layers have been adsorbed, the more is the actual thickness of the whole multilayer assembly exceeding the depth of penetration ($dp \approx 0.5\mu m$), which is the distance from the surface, at which the electric field strength (E) interacting with the transition dipole moments (μ) of the adsorbed molecules has been exponentially decayed to a factor of 1/e with respect to its value at the IRE surface. Hence, at a certain multilayer thickness the band area A (proportional to $(E\,\mu)^2$) due to polyelectrolyte functional groups of the multilayer assembly is not increasing any longer, though additional single layers have been adsorbed. More precisely, the integrated ATR-IR band area scales by a function of the type K(1-exp(-L*N)), which may be related to the 'effective thickness' introduced by Harrick[4], with the adsorption step N, whereby L is a recibrocal thickness parameter and K a constant. A detailed analysis of ATR-FTIR layer-by-layer adsorption data will be presented elsewhere[5].

Additionally, we observed an increase of the negative ν(OH)-band (3700-3000 cm^{-1}, scaled by 1/10) of water in dependence of the adsorption step, since by layer growth the neighboring water/solid interface in the sample compartment was subsequently moved away from the substrate surface in comparison to the unmodified surface in the reference compartment. Furthermore, by the change of the polyelectrolye components oscillating intensity variations of the ν(CO)- and the ν(OH)-band could be registered. This suggests alterations of the surface accessibility of water in dependence on the outermost adsorbed polyelectrolyte, having formed a complex with the previously adsorbed polyelectrolyte.

Protein adsorption at polyelectrolyte multilayers

For the study of surface bioadhesion protein adsorption is estimated as the crucial first step of a complex adhesion sequence when foreign inorganic or polymer surfaces interact with the aqueous biological environment[6]. Hence, we performed adsorption experiments of the plasma protein human serum albumine (HSA) at the multilayer modified surface, in order to evaluate bioadhesion properties on a rather model level which might be relevant for the processing of medical devices or food containers.

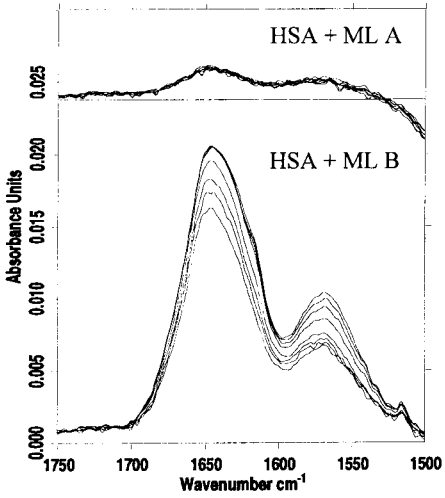

Fig. 3: Time dependent ATR-FTIR-spectra recorded after t = 5, 10, 15, 25, 85, 145, 205, 295 min on the adsorption of human serum albumine (HSA, 1 mg/ml, pH = 7.4) at plasma treated Si-IREs, which have been modified by the multilayer systems ML A (top) [(PDA/PMAPP)$_3$] and ML B (bottom) [(PDA/PMAPP)$_2$+PDA], respectively.

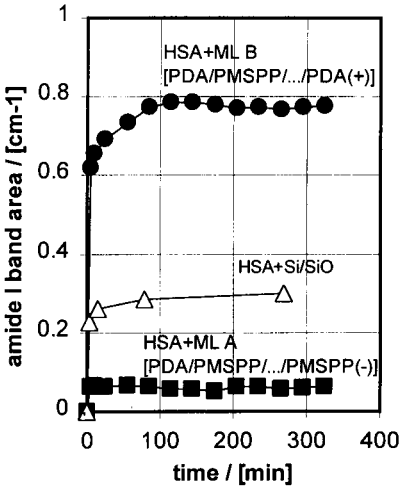

Fig. 4: Adsorption kinetics of HSA (1 mg/ml, pH = 7.4) at the Si-IRE-surface coated by the multilayer systems ML A (bottom) and ML B (top). For comparison the adsorption kinetics at the bare Si-IRE (middle) is shown.

In a recent publication we generally pointed out, that multilayers of oppositely charged polyelectrolytes having the polyanion in the outermost layer could repel like charged proteins and exhibit therefore certain protein inert (non fouling) properties[3]. To further prove this

statement, we generated two different multilayer (ML) arrangements, i.e. [(PDADMAC/PMAPP)$_3$] (ML A) and [(PDADMAC/PMAPP)$_2$+PDADMAC] (ML B), whereby ML A exhibited a polyanion as the outermost layer, whereas the outermost layer of ML B was made up by the polycation.

At the outermost layer of these two MLs the adsorption of HSA (IEP = 4.7) from a buffered solution (pH = 7.4) was measured, respectively, in the *in-situ*-ATR sorption cell. Accordingly, in Fig. 3 the amide I band region of ATR-FTIR spectra, recorded during the exposure of ML A (top) and ML B (bottom) to an HSA solution is shown. Since the intensity of the amide I band scales directly with the protein adsorbed amount we have a quantitative measure on HSA adsorption at these two different MLs, enabling also the determination of surface coverages[7]. Accordingly, the integrated amide I band areas of the on-line recorded ATR-FTIR spectra are shown in Fig. 4. Significantly, for ML B we obtained an adsorbed amount about 10 times higher compared to the adsorbed amount at ML A. For comparison the adsorbed amount at the unmodified plasma treated Si-IRE (Si/SiO_x-surface) is also shown. Although a certain entanglement between the polyelectrolyte layers is claimed[1], this clearly demonstrates that the outermost adsorbed layer, mainly determines the charge character of the multilayer system (i) and that the charge repulsion towards the negatively charged HSA (see IEP given above) is the main contribution for the protein inertness of ML A. (ii). Oppositely, ML B is able to bind HSA to a higher extent by electrostatic attraction (iii) due to its positively charged surface. For the bare unmodified SiO_x-surface we obtained an adsorbed protein amount, which lies between that for the two oppositely charged surfaces, which could be explained by the minor negative surface charge. Furthermore no significant conformational changes of the adsorbed HSA in dependence of these three different surfaces (ML A, ML B, bare SiO_x), which could have been rationalized by amide I lineshape analysis[8], were observed.

For comparison with a former study[3] the HSA adsorption behavior at the multilayer system composed of poly(ethylene imine) (PEI) / poly(acrylic acid) (PAC), which was deposited on CO_2-plasma treated PP-films, was further investigated. Here we deposited either 4 PEL layers [(PEI/PAC)$_2$] (ML´ A) or of 5 PEL layers [(PEI/PAC)$_2$+PEI] (ML´ B). Anlogously to Fig. 4, we obtained from Fig. 5, that there is only a minor adsorbed HSA amount for ML´ A, which exposes an outermost adsorbed PAC layer, as it was described therein[3] and that again there is a high affinity of HSA towards the outermost positively charged PEI layer of ML´ B. Similarly to the bare Si-IRE, for the unmodified plasma treated PP-film there was a level of

adsorbed HSA amount, which ranged in between the positive and the negative surface charge modifications due to less negative surface groups.

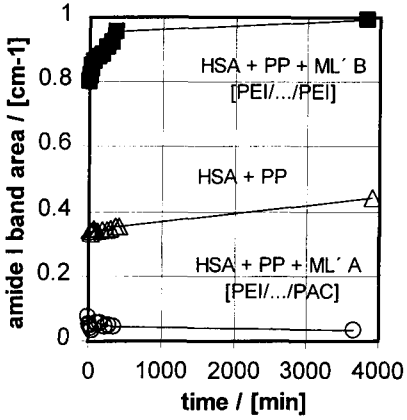

Fig. 5: Adsorption kinetics of HSA (1 mg/ml, pH = 7.4) at Si-IREs coated by CO_2-plasma modified PP-films modified by the multilayer systems ML´ A or ML´ B (see text), respectively. For comparison the adsorption kinetics at the bare PP-film on the Si-IRE is shown.

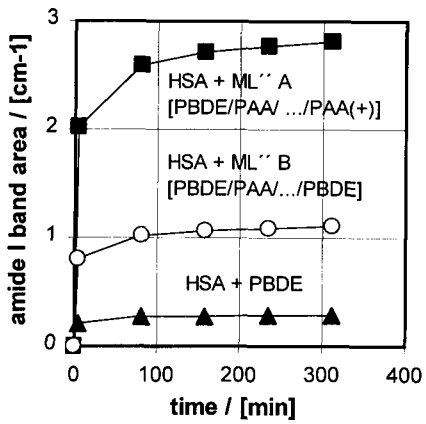

Fig. 6: Adsorption kinetics of HSA (1 mg/ml, pH = 7.4) at Si-IREs modified by the multilayer systems ML´´ A or ML´´ B (see text), respectively. For comparison the adsorption kinetics at the bare PBDE-film on the Si-IRE is shown.

For further studies additional work has to be done to elucidate if comparable charge selective HSA adsorption does occur for any type of oppositely charged polyelectrolyte pairs suited for multilayer formation. In that context a third type of multilayers of polyelectrolytes, which potentially were used for encapsulation systems aiming at the immuno isolation of pancreatic islet cells[9], were deposited as recently published[10]. Thereby, analogously to the ML and ML´ systems, 4- and 5-layer systems using sodium alginate/cellulose sulfate (SA/CS) and poly(methylene-co-guanidinium chloride) (PMCG) were generated. Similarly, a significant low adsorbed amount for the 5-layer system ending up with SA/CS and a 5 times higher adsorbed amount for the 4-layer system exposing the polycation PMCG was obtained[10].

Protein adsorption at chemo-reactive polymer multilayers

In addition to the consecutive deposition of polyelectrolyte multilayers, whereby the main driving force is the electrostatic interaction between oppositely charged segments (electrosorption), also multilayers consisting of polymers, whose reactive groups can cause chemical bonding, were deposited by alternate chemisorption. As an example for such chemo-reactive polymer pairs we have chosen poly(butadienepoxide) (PBDE), whose chemisorption at silica surfaces and further modification was reported recently[11], and poly(allylamine) (PAA).

The according stepwise chemisorption was also recorded by ATR-IR spectroscopy and the data will be published elsewhere[12]. Here again two multilayer systems (ML'' A, ML'' B) composed of PBDE and PAA are focussed on, whereby the MLs are prepared by alternately dipping the IRE in a PBDE and PAA solution ($CHCl_3$) to generate either the arrangement PBDE/PAA/PBDE/PAA/PBDE (ML'' A) or PBDE/PAA/PBDE/PAA/PBDE/PAA (ML'' B). In Fig. 6 the according HSA adsorption data, which was measured analogously to above, is shown. Similarly to the polyelectrolyte multilayers we have an increase of HSA adsorbed amount for ML'' B, which is due to the ammonium groups of outermost PAA generated in the presence of water. Whereas for ML'' A having PBDE on top, there is a certain decrease of the HSA adsorbed amount, since the positively charged groups at the surface might be diminuished. However the fact that there is still a considerable amount of bound protein might be explained by both the incomplete coverage of the underlying PAA layer (i) and hydrophobic interaction between the CH_2 moieties of PBDE (ii).

CONCLUSION

Conclusively, multilayers are easy-to-handle, physically stable and tunable model systems, from which we can learn more on the selectivity of bioadhesion processes. Thereby, the affinity to HSA could be controlled by the choice of the last adsorbed polymer layer, which should be relevant for applications in the food and biomedical industry particularly aiming at both inert as well as selective protein binding surfaces driven by electrostatic interaction.

For basic research further work is dedicated on the one hand to structural variation of the polyelectrolytes due to charge density, weak/strongness and hydrophobic contribution. On the other hand we started to apply further model proteins of different charge, size and hydrophobic properties[13]. In how far polyelectrolyte multilayers are a proper model for testing of microcapsules has to be further worked out.

Multilayers composed of chemo-reactive polymer pairs like PBDE / PAA reveal surface selectivity for HSA, which is comparable to the polyelectrolyte based systems.

Yet speculatively, model proteins, which are proven to be highly selective and sensitive for surface charge and functionality, may be used as probe molecules for any surface of interest.

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