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Protein adsorption to planar electrochemical sensors and sensor materials*

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Abstract: In electrochemical sensing devices, aimed for acute and chronic in vivo application, the active surface of the sensor is often negligible compared to the overall surface area of the device in contact with the biological host. Consequently, to minimize the perturbation of an implanted sensor on the in vivo environment the chemical composition and surface texturing of the complete device (the active sensor, sensor substrate, and "accessories") have to be considered. In our work, the adsorption of three abundant proteins (albumin, IgG, and fibrinogen) was determined quantitatively on untreated and modified sensor substrates and sensing membrane surfaces. In this study, a flexible polyimide-based material (Kapton®) was used as sensor substrate with or without an amorphous diamond-like carbon (DLC) or an amorphous oxygen-containing DLC (*o*-DLC) coating. The ion-sensitive membranes were cast from high-molecular-weight (HMW) or carboxylated poly(vinyl chloride) (PVC) and were doped with increasing concentrations of highly hydrophilic poly(ethylene oxide) (PEO). The potentiometric characteristics of the potassium-selective membranes cast with up to 6 % PEO were the same as those without PEO. However, the PEO-modified PVC membranes elicited a large amount of protein adsorption, especially in terms of albumin.

INTRODUCTION

Requirements for simple, accurate, and reliable measurements in medicine and life sciences were often the driving forces behind the development of electrochemical sensors. In recent years, the macroscopic size, membrane-based chemical and biosensors have been miniaturized and technologies commonly used in the microelectronic industries including thick-film screen printing, thin-film photolithography are gradually replacing conventional sensor manufacturing [1]. The integration of multiple sensors into microscopic flow channels or onto single-use chips allows multicomponent analysis in a few microliters of sample and projects the possibility of acute or chronic in vivo applications [2]. However, despite the need for continuous monitoring of blood gases (CO_2, O_2) and electrolytes (H^+, K^+, Na^+, Ca^{++}, etc.) in critically ill patients, such devices did not gain widespread applications due to the biofouling of the implanted devices.

Biofouling is the accumulation of proteins, cells, and other biological materials on an implanted surface [3]. Proteins rapidly adsorb to available solid–liquid interfaces. The adsorption of proteins to planar surfaces often changes the protein's conformation, which can lead to functional inactivation and to the formation of a gel-like layer of denatured protein [4]. This gel-like layer is considered to be responsible for a number of deleterious processes referred to as membrane biofouling, fibrous encapsulation, and membrane biodegradation, which ultimately lead to sensor failure in vivo.

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To prevent membrane biofouling and to control the biological responses to an implanted sensor, complementary strategies could be used. Surface modifications and special coatings are widely applied for reducing protein adsorption and platelet attachment [5–11]. For controlling the tissue responses, local drug delivery systems can be implemented [12]. The tissue responses can also be influenced by optimal surface topography (roughness, texture, and porosity) [13]. However, it is also essential to preclude the possibility of leaching membrane components into the contacting tissue that can induce inflammatory responses [14,15]. The methods for reducing biosensor membrane biofouling have been reviewed recently by Wisniewski [16].

The selection of the most appropriate coating is extremely challenging due to the different mechanisms of membrane biofouling and because not all modifications or membrane coatings are compatible with the base transducer or the sensing membrane. With amperometric sensors (like the glucose sensor), an outer protective layer having ion-exchange properties can boost selectivity and control the analyte diffusion rate determining the sensitivity and the dynamic range of the sensor [17]. However, when ion-selective electrodes are coated with membrane layers having ion-exchange properties, the selective potentiometric response may be completely impaired [18].

The sensing membrane of most ion-selective electrodes (ISEs), with relevance for biomedical applications, is cast from highly plasticized high-molecular-weight (HMW) poly(vinyl chloride) (PVC), which is considered poorly blood-compatible [19]. To improve the blood compatibility of the sensing membrane, there were several attempts to replace the PVC membrane with novel, more biocompatible polymers, for example, polyurethane derivatives, poly(ethylene oxide) derivatives [20], silicone rubber, poly(2-methoxyethyl) acrylate, etc. [21]. Others have tried to modify the sensing membrane with covalently attached heparin [22] and by implementing NO-releasing polymer films [23] for preventing platelet adhesion and activation. Hydrogels are also widely used for masking the underlying surfaces. However, when microfabricated electrodes are implemented to in vivo applications, the active area of the sensor is often negligible compared to the overall surface of the implanted device. Consequently, with respect to the overall biological response of the host, it is not enough to consider the physical and chemical characteristics of the materials that make up the sensor. The size, geometrical shape, mass, chemical composition, and surface texturing of the sensor supports and "accessories" are all critical.

To function in vivo, the implanted device must minimally perturb the in vivo environment (biocompatible), and the in vivo environment must minimally perturb the performance of the sensor (sensocompatible). From the analytical chemist's perspective, the second requirement may seem more important. However, the detrimental effects of protein adsorption, cellular adhesion, inflammatory reactions, and fibrous capsule formation can only be minimized if the first requirement is fulfilled.

In our group, microfabricated ion-selective electrodes for in vivo applications are formed on flexible Kapton® substrates [24,25]. These planar ion-selective electrodes were selected as model sensors in this study. Related to the importance of protein adsorption in the activation of the host's immune system, the amount of three abundant proteins (albumin, IgG, and fibrinogen) adsorbed to the sensor substrate and sensor membrane surfaces were evaluated quantitatively. In the frame of the study, both the sensor substrate and a sensor membrane were modified for better biocompatibility, and the protein adsorption was evaluated in the light of these modifications. The base supporting polyimide film (Kapton) was modified with two different hydrophilic coatings (amorphous oxygen-free diamond-like carbon, DLC, and amorphous oxygen-containing DLC, *o*-DLC). The ion-sensitive membranes were cast from high-molecular-weight and carboxylated poly(vinyl chloride) (PVC-HMW and PVC-COOH, respectively). The PVC membranes were doped with increasing concentrations of highly hydrophilic poly(ethylene oxide) (PEO). In addition, we compared the protein adsorption on PVC-HMW membranes that were cast with or without the potassium selective ion-selective ionophore, BME-44.

PEO has been selected as an ion-selective membrane additive because it is well recognized as a particularly effective polymer to produce blood-compatible surfaces due to its low interfacial free energy with water and molecular conformation, hydrophilicity, high surface mobility, and steric stabilization effects [26]. It has been incorporated into different polymeric matrices as a possible mean of in-

creasing the hydrophilicity [11,27–29] and for improving the blood compatibility of ion-selective materials. In their attempt to clarify the effect of PEO additives on intra-arterial ion-selective electrode performance, Espadas-Torre and Meyerhoff [11] showed that platelets formed a densely packed aggregate on the surface of the conventional PVC-based pH and potassium-selective membranes. Unfortunately, the membranes doped with PEO copolymers displayed a large decrease in slope and selectivity, when placed in an 8 % (w/v) bovine serum albumin solution, compared to membranes not containing the copolymer.

EXPERIMENTAL

Sample preparation

In our studies untreated Kapton films (DuPont), DLC-coated Kapton films and *o*-DLC-coated Kapton films were evaluated for their protein adsorption properties as sensor substrate materials. Both types of DLC-coated Kapton films were prepared at room temperature by plasma-enhanced chemical vapor deposition (PECVD), and were used as received from Premitec Inc. Both surfaces are considered highly hydrophilic compared to the untreated Kapton surfaces, which was quantified by contact angle measurements performed by Premitec, Inc. with a goniometer (Model 100, Ramé-Hart, Inc.). The experimentally determined contact angle data for the studied sensor substrate and sensing membrane surfaces are summarized in Table 1.

Table 1 Experimentally determined contact angles of H_2O on different sensor substrate and sensing membrane surfaces and the adsorbed amounts of proteins (albumin, IgG, and fibrinogen in ng/cm^2) on the same surfaces. The mean values are given with their standard deviations. The data were calculated from a minimum of three (n = 3) and up to six (n = 6) parallel measurements. In the case of small contact angles, only a range or an approximate value could be determined.

Materials	Membrane code	Contact angles	Protein adsorbed		
		Mean ± SD	Albumin ng/cm^2 mean ± SD	IgG ng/cm^2 mean ± SD	Fibrinogen ng/cm^2 mean ± SD
PE		87.0 ± 2.7	147 ± 17	208 ± 27	95 ± 32
Kapton untreated		70.6 ± 2.1	69 ± 39	116 ± 6	187 ± 15
DLC-treated Kapton		10 to 15	401 ± 81	1805 ± 247	609 ± 120
o-DLC-treated Kapton		~10	97 ± 49	199 ± 15	100 ± 68
PVC-HMW	A	83.6 ± 2.6	208 ± 27	307 ± 36	68 ± 25
PVC-COOH	B	66.2 ± 2.7	302 ± 30	285 ± 6	209 ± 18
PVC-HMW/BME-44	C	78.3 ± 2.1	1131 ± 92	301 ± 13	118 ± 26
PVC-HMW/3 % PEO	D	62.6 ± 1.2	3333 ± 144	604 ± 60	384 ± 53
PVC-HMW/6 % PEO	E	64.3 ± 1.2	4054 ± 348	757 ± 63	473 ± 65
PVC-HMW/10 % PEO	F	64.6 ± 0.6	4060 ± 364	920 ± 76	445 ± 44

The protein adsorption data of the sensor substrate materials were compared to protein adsorption data measured on polyethylene (PE) primary reference material from NIH. For the experiments, circular disks with a diameter of 1.2 cm were cut from these materials and were placed in an incubator at 37 °C and allowed to equilibrate. To study the protein adsorption on the ion-selective membrane materials, the 1.2-cm-diameter Kapton disks were dip-coated in specific membrane cocktails. The membrane cocktails contained a mixture of ion-selective membrane ingredients (polymeric material, plasticizer, ionophore, and lipophilic salt additive) dissolved in tetrahydrofuran (THF, Fisher Sci.). As polymeric material, PVC-HMW, carboxylated PVC (PVC-COOH) (Fluka), or a PEO (200 000 MW;

Scientific Polymer Products, Inc.) doped PVC-HMW mixture was used. After the coated surface became dry with the first polymeric membrane layer, the dip-coating procedure was repeated.

Membrane and sensor preparation

The composition of the membrane cocktails is listed in Table 2. A plasticizer, 2-nitrophenyl octyl ether (*o*-NPOE) (Fluka, 73732), was added to each membrane cocktail in 1:2 polymer to plasticizer ratio (w/w %). The membranes C, D, E, and F had different PEO content but contained the same amount of potassium-selective ionophore BME-44 [30–32] (potassium ionophore III, Fluka 60397) and lipophilic salt additive, KT_PC1PB {potassium tetrakis [3,5-bis (trifluromethyl) phenyl] borate; Fluka, 60588}. The amount of KT_PC1PB was the same in each membrane. It was 50 mol % with respect to the ionophore. The membrane compounds were dissolved in 2 ml of tetrahydrofuran (THF, Fisher Sci.) and were used for the dip-coating or were cast into glass rings (30 mm diameter) fixed on a glass plate. The PEO-containing cocktails had to be sonicated for 15 min for complete dissolution.

Table 2 The compositions and the calibration properties (slopes and detection limits) of K^+ selective membranes on the surface of which the adsorption of different proteins were evaluated quantitatively. The slope values represent the mean ± standard deviation (SD) of a minimum 3 measurements observed over a 1-month period. All membranes were cast with 200 mg of the plasticizer *o*-NPOE.

Membrane	PVC-HMW mg	PVC-COOH mg	PEO mg	BME-44 mg	KT_PC1PB mg	Slope (10^{-1}–10^{-5} M) mV/decade	Slope (10^{-1}–10^{-5} M) mV/decade	DL (log c_K)
A (PVC-HMW)	100	–	–	–	–	–	–	–
B (PVC-COOH)	–	100	–	–	–	–	–	–
C (PVC-HMW/BME-44)	100	–	–	3	1.34	56 ± 0.7	58 ± 0.3	–5.29
D (PVC-HMW + 3 % PEO)	90	–	10	3	1.34	56 ± 0.7	57 ± 0.7	–5.25
E (PVC-HMW + 6 % PEO)	80	–	20	3	1.34	54 ± 0.7	57 ± 1.3	–5.17
F (PVC-HMW + 10 % PEO)	70	–	30	3	1.34	30 ± 0.7	32 ± 1.7	–5.20

The glass rings were covered with another glass plate to slow THF evaporation. After the complete evaporation of the THF, 7-mm-diameter disks were cut from the resulting 200-µm-thick membranes. These disks were placed into Philips liquid membrane electrode bodies (IS-560, Moller Glassblaserei, Zürich, Switzerland) for potentiometric characterization.

Proteins and reagents

Working aliquots of human fibrinogen, immunoglobulin G (IgG) (Calbiochem-Novabiochem Corp.), and albumin (Sigma-Aldrich) were prepared in phosphate-buffered saline (PBS: 7.2 mM $Na_2HPO_4{\cdot}7H_2O$; 2.8 mM $NaH_2PO_4{\cdot}H_2O$; 0.14 NaCl; pH 7.4). Platelet poor plasma (PPP) was prepared from a stock of whole blood pooled from seven donors. The amount of albumin (33.9 mg/ml) in the PPP was determined by a total albumin assay (Sigma Diagnostics), and the amount of fibrinogen (2.5 mg/ml) and IgG (11.5 mg/ml) present in the PPP was estimated according to typical values.

Protein adsorption

The specimens were placed in 2.5-ml polystyrene cups and then allowed to equilibrate at 37 °C. A 30 % plasma solution, which included ^{125}I-labeled protein, was prepared by the addition of the appropriate amount of ^{125}I-labeled protein needed to create an ideal level of radioactivity (1000 counts per minute, CPM). Three 10-µl aliquots of the 30 % plasma solution were placed in the Packard® Cobra II gamma

counter to determine the specific activity of the plasma solution. The 30 % plasma solution was then allowed to incubate at 37 °C.

For each experiment, 1.5 ml of the prepared 30 % plasma solution was added to each polystyrene cup containing the studied specimen and incubated for 1 h at 37 °C. The specimens were then removed from the plasma solution and dip-rinsed three times in PBS to remove nonadsorbed proteins. The rinsed samples were then placed in polystyrene counting tubes to determine the amount of radioactivity on the studied surfaces. The amount of bound protein was then calculated using the specific activity of the 30 % plasma solution and the planar surface area of each specimen.

One-way analysis of variance (ANOVA) and the Student–Newman–Keuls test were used to determine if there was a statistically significant difference between the adsorbed protein amounts on the surfaces of the different materials involved in this study. The protein adsorption properties of the studied surfaces were considered to be significantly different when $p < 0.05$.

Electrode characterization

An Orion® Model 90-02 double junction Ag/AgCl reference electrode with 1 M LiOAc outer filling solution was used as a reference electrode in all experiments. A Lawson Labs, Inc. (Malvern, PA) 16-channel data acquisition system, driven by L-EMF DAQ, a LabView program, was used to record the measured cell voltage value. All measurements were made at room temperature in an air-conditioned laboratory.

During the potentiometric characterization of the sensors, the response slopes, the detection limit, the potential stability, reproducibility, and the selectivity coefficients were evaluated for each sensor according to IUPAC recommendations [33]. The sensors were calibrated in KCl standard solutions with concentration levels ranging from 10^{-1} to 10^{-6} M. Since the physiological concentration range of the K^+ ion lies within 10^{-2} and 10^{-3} M, the reproducibility measurements were evaluated at these two concentrations. The selectivity coefficients ($K_{i,j}^{pot}$) were determined by the separate solution method using 10^{-1} M concentration of KCl, NaCl, NH_4Cl, and $CaCl_2$ solutions [33]. The extended Debye–Huckel equation was used to calculate the mean activity coefficients, and the Henderson equation was used to account for the changes that occur in the liquid junction potential [34].

RESULTS

Analytical characterization of the membranes

The calibration slopes and detection limits of the studied electrode membranes are summarized in Table 2. The slopes of the calibration curves for membranes C, D, and E were near Nernstian in the concentration range between 10^{-1} and 10^{-5} M KCl. The detection limit for all the ISEs were similar ($\log c_{DL} = -5.23 \pm 0.05$). The reproducibility of the measured potentials in the same solution was between 0.1 and 0.2 mV for all membranes, when the samples were alternated between 10^{-2} and 10^{-3} M KCl ($n = 10$). Similarly, there was no significant difference in the stability of the measured electrode potentials when the electrodes were kept in a solution of constant composition (10^{-3} M KCl). In a 20-h experiment, the drift was determined as ~1.1 mV/h. The membrane composition did not show any effect on the drift.

The logarithms of the selectivity coefficients ($\log K_{i,j}^{pot}$) for the studied membranes are summarized in Fig. 1. It shows that with the increasing amounts of PEO incorporated into the PVC-HMW matrix the selectivity coefficients of the BME-44-based potassium electrode toward all the studied interfering ions get somewhat worse. However, up to 6 % of PEO, this deterioration is modest. The selectivity coefficients of the electrodes with 3 and 6 % PEO content in their membrane are appropriate for the selective potassium determination in biological samples, like whole blood, blood serum, and

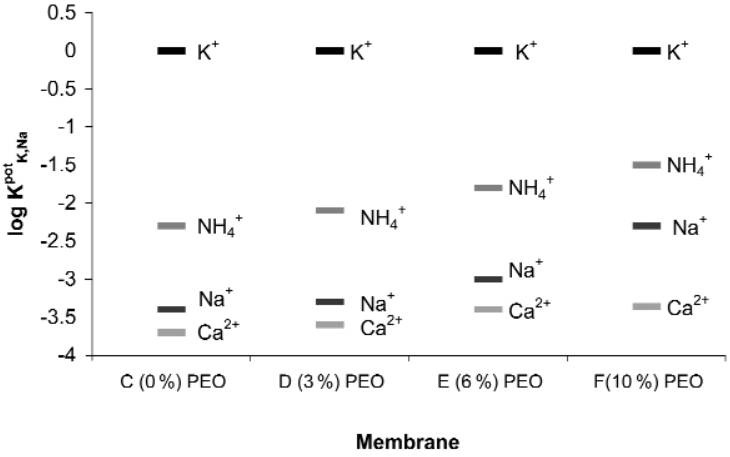

Fig. 1 Selectivity coefficients for K^+ selective membranes with and without PEO doping. The 3, 6, and 10 % PEO content refers to the percent PEO in the ion-selective membrane. The membrane compositions are given in Table 2. Data represent the logarithms of the selectivity coefficients $K_{i,j}^{pot}$ for the primary K^+ ion and the interfering ions Na^+, Ca^{2+}, and NH_4^+.

plasma. The selectivity coefficients of these membranes are similar to the data reported in literature on BME-44-based membranes [31,35].

Protein adsorption

The amount of albumin, IgG, and fibrinogen adsorption to the different sensor substrate and sensing membrane materials were quantified in order to predict overall hemocompatibility of an implanted potentiometric sensing device. All adsorption data reported in this study represent the mean ± standard deviation of the adsorbed amounts in ng/cm^2. The adsorption profiles for albumin, IgG, and fibrinogen to the selected biosensor materials are summarized in Figs. 2–5 and Table 1.

Albumin

As can be seen in Fig. 2 and Table 1, the untreated Kapton adsorbed the least amount of albumin followed by the *o*-DLC-coated Kapton. Both the *o*-DLC- and DLC-treated Kapton adsorbed more albumin, however, in the case of the *o*-DLC coating this increase was not significant. When the adsorbed amounts of albumin on the two surface-treated Kapton specimens are compared, it shows that the DLC-coated Kapton adsorbed significantly ($p > 0.05$) larger amount of albumin, although the contact angles measured on these surfaces were very similar.

The difference in the adsorbed amounts of albumin on the surface medical-grade polyethylene standard, the unmodified and *o*-DLC-coated Kapton specimens, and on the surfaces of the *o*-NPOE-plasticized PVC-HMW and PVC-COOH dummy membranes (membranes without the ion-selective ionophore) was statistically not significant ($p > 0.05$). But, somewhat unexpectedly, there was a significant difference between the adsorbed albumin to the PVC-HMW membranes with (membrane C) and without (membrane A) the potassium-selective ionophore BME-44 ($p < 0.01$).

The PEO doping of the PVC-HMW membranes significantly increased the adsorbed albumin amounts. Membranes cast with more PEO adsorbed more albumin, however, the difference in the adsorbed amounts was not significant between membranes E and F (Table 1). The measured values were about one order of magnitude larger (between 3333 and 4060 ng/cm^2) than with any of the other materials. Although the PEO content in the membranes D, E, and F ranged only between 3 and 10 % of the total membrane weight, the adsorbed albumin amounts were three to four times larger than on membrane C, with very similar composition, but without PEO.

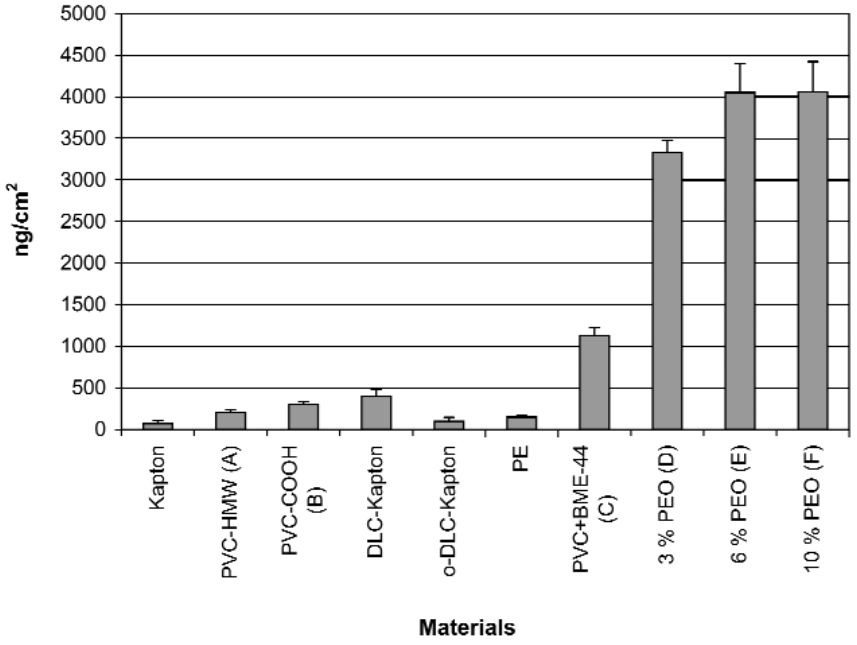

Fig. 2 Albumin adsorption to selected biosensor materials. Data are reported as the mean ± SD. An $n = 6$ for Kapton, PVC-HMW, PVC+BME-44, PVC-COOH and DLC-Kapton, $n = 5$ for *o*-DLC-Kapton. and an $n = 3$ for PE.

IgG

Similar to the albumin adsorption, the untreated Kapton adsorbed the least amount of IgG (116 ng/cm^2), and the PEO-doped PVC membranes (membranes D, E, and F) adsorbed relatively large amounts of IgG. The adsorbed amounts of IgG were about two to three times more than on other studied surfaces,

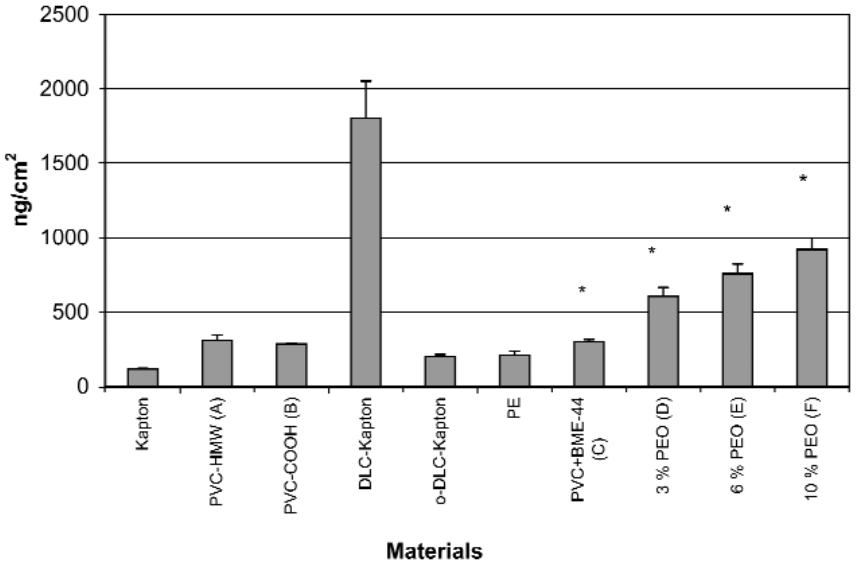

Fig. 3 IgG adsorption to selected biosensor materials. Data are reported as the mean ± SD with $n = 3$ and $n^* = 6$.

including membrane C. However, in this study, the DLC-coated Kapton adsorbed the largest amount of IgG (1805 ng/cm^2). The values are shown in Fig. 3 and Table 1.

In summary, the medical-grade PE adsorbed an amount of IgG similar to all materials ($p > 0.05$) except the DLC-coated Kapton and the three PEO-doped PVC-HMW samples ($p < 0.01$). The measured values on the PEO-doped PVC samples increased proportionally with their PEO content and differed significantly from each other ($p < 0.01$). The data also show that the IgG adsorption was similar on the PVC-HMW, PVC-COOH, and the ionophore-loaded PVC-HMW membranes ($p > 0.05$).

Fibrinogen

As shown in Fig. 4 and Table 1, the major trends experienced with fibrinogen adsorption were similar to those found for albumin and IgG adsorption. The PVC-HMW membrane adsorbed the least amount (69 ng/cm^2), however, the fibrinogen adsorption was similar on the *o*-DLC-treated Kapton, the BME-44-loaded PVC-HMW membranes (membrane C) and on the medical-grade PE materials. The measured differences were statistically not significant ($p > 0.05$). On the other hand, the DLC-coated Kapton adsorbed the most fibrinogen (610 ng/cm^2), and the adsorbed amount was significantly larger than with any of the other materials tested in this study ($p < 0.01$). Following the DLC-coated Kapton, the PVC-HMW membranes with incorporated PEO (membranes D, E, and F) showed the most fibrinogen adsorption. The adsorbed amounts on these modified PVC materials were significantly larger than on all other studied materials, except the DLC-coated Kapton ($p < 0.01$).

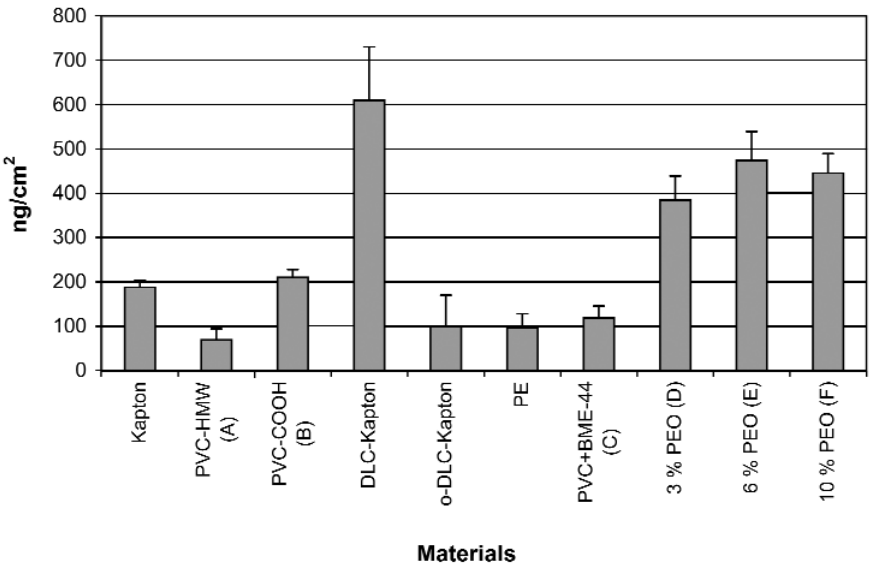

Fig. 4 Fibrinogen adsorption to selected biosensor materials. Data are shown as the mean values ± SD and an $n = 6$ for all materials.

The total amount of protein that adheres to the sensor's membrane, regardless of their individual effect on biocompatibility, can affect the analyte transport characteristics. Thus, to compare the total amount of adsorption between materials, an adsorption profile for all materials is summarized in Fig. 5. The data show that all three PVC membranes with incorporated PEO adsorbed rather large amounts of protein. In contrast, the adsorbed amounts of different proteins on the untreated and *o*-DLC-coated Kapton surfaces and the PVC-HMW and PVC-COOH membranes were significantly smaller.

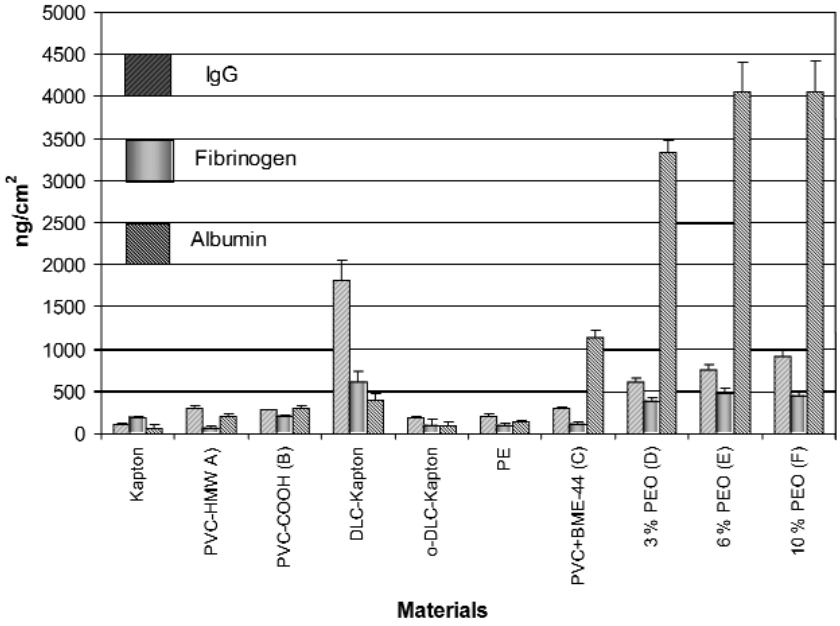

Fig. 5 Adsorption profile for selected biosensor materials. The data represent the mean ± SD with a minimum $n = 3$ for each experiment.

DISCUSSION

Biocompatibility

In this study, the amount of albumin, IgG, and fibrinogen adsorbed to sensor substrate and sensing membrane materials, commonly used to manufacture planar ion-selective sensors, were determined quantitatively. By comparing the protein adsorption on the different sensor support and sensing membrane materials we intended to find some general guidelines for reduced biological responses or improved biocompatibility of implanted sensing devices. Based on the general preference for hydrophilic surfaces in biomedical applications, we were interested in the influence of the hydrophobic/hydrophilic character of a surface on the adsorption of the most abundant proteins. The hydrophobic/hydrophilic character of the studied surfaces was quantified by contact angle measurements (Table 1). For changing the hydrophobic character of the unmodified Kapton films, the Kapton surfaces were coated by a highly hydrophilic *o*-DLC or DLC layers. Both coatings decreased the experimentally determined contact angles from Θ ~70° to about $\Theta < 15°$. However, in spite of the similarities in the contact angles, these two modified Kapton surfaces attracted proteins completely differently. The *o*-DLC-modified Kapton surface was always among the materials with the least protein adsorption. In contrast, the DLC-coated Kapton surface adsorbed the most from IgG, and fibrinogen and the adsorbed albumin amount was also five to six times more than on the untreated Kapton or the *o*-DLC-coated Kapton. Interestingly, the untreated and the *o*-DLC-coated Kapton surfaces behaved similarly to each other and to the medical-grade PE. These data suggest that the contact angles alone are not appropriate for predicting the protein adsorption on a given surface. The interaction of the individual proteins with a surface of complex chemical composition cannot be expressed with this very general surface property. On the other hand, besides the total adsorbed amount, it would be important to know the strength of the interactions between the studied surface and the proteins. It is assumed that the interactions are weaker when the surfaces are made more hydrophilic.

To clarify the unexpectedly large adsorbed amounts on the surface of DLC-coated Kapton, further studies, involving platelet activation, might be necessary. Scanning electron or atomic force mi-

croscopic studies of the surface could indicate that the effective surface was greatly underestimated, and the calculations based on the geometrical surface area led to the apparently large adsorbed amounts.

Based on the central role of fibrinogen in platelet adhesion, it was interesting to learn that the fibrinogen adsorption was the smallest on the *o*-NPOE-plasticized PVC-HMW membrane. The amount of fibrinogen adsorbed to the PVC-HMW surface was less than that determined on the surface of the medical-grade PE standard. The fibrinogen adsorption remained relatively small after the same membrane has been compounded with the ion-selective ionophore and the lipophilic salt additive KT_PClPB. These findings apparently contradict the generalized view that considers plasticized PVC membranes of poor blood compatibility [19].

Small changes in the chemical composition of the membranes may induce large changes in the adsorbed protein amounts. These changes may be specific to a given class of protein. The carboxylated PVC membrane with 1.8 % carboxylic group concentration in the membrane adsorbed a significantly larger amount of fibrinogen than the PVC-HMW membranes (Table 1). Similarly, the PVC-HMW membrane loaded with 1 % potassium-selective ionophore BME-44, adsorbed about five times the amount of albumin than the same membrane, but without the ionophore. (Compare the adsorbed albumin amount on membranes A and C in Table 1.)

The incorporation of PEO into the ion-selective membranes was performed in hopes of increasing the hemo/biocompatibility of these membranes without impairing their analytical characteristics. As we showed above, the analytical characteristics of the membranes with up to 6 % PEO (up to 20 % with respect of the polymer) were similar to those cast with the conventional composition. However, all three PEO-modified membranes adsorbed much larger amounts of albumin than the other materials. Similarly, the adsorbed amounts of fibrinogen and IgG were very high on PEO-modified PVC membranes. As the PEO content was increased from 3 to 6 %, the adsorbed amounts increased significantly for all three proteins. When the PEO content was increased further (up to 10 %), the adsorbed albumin and fibrinogen remained constant, but the amount of IgG increased again.

CONCLUSIONS

The results obtained in this study show the complexity of biosensor optimization. Since the potentiometric characteristics of the potassium selective membranes with 3 and 6 % PEO content were practically the same as those with conventional membrane composition they appeared to be attractive candidates for in vivo applications. However, all three PEO-modified PVC membranes elicited a large amount of protein adsorption, especially in terms of albumin. The adsorbed amount of albumin was about one order of magnitude larger than the measured amounts on the other examined materials. This large amount of adsorbed albumin may be disadvantageous if it forms a gel-like barrier at the sensor surface, which slows down the analyte transport and increases the response time of the sensor. However, it may also have advantages because the adsorbed albumin layer may increase the hemocompatibility of the surface, which could be beneficial in terms of decreased platelet deposition, a key step in thrombosis. Espadas-Torre and Meyerhoff [11] showed a decrease in platelet attachment on PEO-treated surfaces. Their findings can be considered as experimental support for the beneficial effect of increased albumin adsorption on PEO-blended membrane surfaces. Further studies are necessary to determine the effect of these adsorbed proteins on the biological response of the host and on the long-term analytical performance of these membranes in biological samples.

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