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Ellipsometric studies of antisera binding onto medical polyurethanes immersed in human plasma *in vitro*

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The plasma protein binding properties of four medical polyurethane materials were investigated. The polymers were Pellethane 2363-80AE, ethanol extracted Pellethane, a poly(ether urethaneurea) with a composition similar to Biomer and containing an acrylic polymer additive, and the same polymer without the additive. The polymers were cast onto silicon wafers and then immersed in human plasma from solution followed by antisera. The deposition of organic material was measured with ellipsometry. All tested polymers displayed low anti-high molecular weight kininogen (α-HMWK), anti-Factor XII (α-F XII), and anti-fibronectin (α-FN) depositions. Polymers containing additives also showed significantly lowered binding of anti-fibrinogen (α-FG). The results show that protein binding to the tested polyurethane surfaces were largely modulated by surface active processing aids, and can be conveniently analysed using antisera techniques.

1. Introduction

A layer of adsorbed blood proteins occurs on a foreign material within seconds of initial blood contact. The nature of the layer is believed to influence the subsequent events whereby the surface can either be passivated or activated for blood clotting [1]. Different hypotheses have been proposed to explain the behaviour of synthetic materials in relation to blood coagulation [2–6]. It is believed that hydrophilic surfaces will show less overall protein adsorption than hydrophobic ones and thus will be less thrombogenic [2]. Alternatively, certain hydrophobic surfaces are believed to preferentially adsorb albumin, thereby passivating the surface for further interactions with the blood components [7–9].

Segmented polyurethanes are widely used in biomedical applications because of their excellent physical and chemical properties, and good blood compatibility [10]. The most commonly used commercial materials are Pellethane 2363 (Dow-Upjohn) and Biomer (Ethicon). Pellethane is a thermoplastic poly(ether urethane) and may be melt processed, while Biomer being a poly(ether urethaneurea), has to be processed by solution casting. Biomer has been used for long-term blood contacting applications such as ventricular assist devices and total artificial heart. Pellethane is used quite extensively for production of catheters and pacemaker leads [11]. The long-term chemical and biological stability of polyether-based polyurethanes are generally good although instances of surface stress-cracking in pacemaker wire coatings prepared from Pellethane have been reported [12, 13] as well as hydrolytic, oxidative, and enzymatic biodegradation [14–17].

Linear poly(ether urethanes) and poly(ether urethane ureas) are relatively non-thrombogenic, as compared to most polymer materials [10]. The reasons for this are unclear. It has been suggested that the phase separated morphology of their surfaces, i.e. the pattern of soft polyether domains and hard urethane or urea domains would have an influence. Many studies have been carried out along these lines [3–5, 9].

Most commercial polymers contain different types of additives, i.e. antioxidants, mould release agents and other types of surface active components. Pellethane, which is manufactured by Dow-Upjohn, is believed to be composed of polytetramethylene glycol (PTMG) and 4,4-methylene bis(phenyl isocyanate) (MDI), and with 1,4-butanediol being used as the chain extender. The polymer generally contains a processing aid consisting of a fatty acid amide [18], which can be removed from the polymer by extraction. The extracted Pellethane exhibits quite different surface properties.

The composition of Biomer is similar to the original Lycra Spandex 127 manufactured by E.I. Du Pont de Nemours & Co. According to a report by Belisle *et al.* [19], the monomers of Biomer have been identified as

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MDI, PTMG 1800, and ethylene diamine. In addition to the base polymer, Biomer was also found to contain a standard antioxidant and a polyacrylic additive, identified as poly(diisopropylaminoethyl methacrylate-co-decyl methacrylate) [19, 20].

In the present study, we have investigated the protein adsorption from blood plasma onto surfaces of Pellethane and a poly(ether urethaneurea) (PUUR) of a composition similar to Biomer. The objectives of the investigation was to evaluate the effects of the above-mentioned additives on the protein (antisera) binding patterns. The polymer surfaces were characterized by receding and advancing water contact angle measurements and X-ray photoelectron spectroscopy (XPS) analyses. The polymer–blood plasma interactions of the surfaces were studied through incubation of the surfaces in a non-flowing citrated human plasma, followed by antisera deposition onto the proteinacous layer. The information may be valuable for further guidance in making thrombo-resistant materials.

2. Materials and methods

2.1. Polymer preparation

Pellethane 2363-80AE, a commercially available biomedical grade segmented polyurethane, was obtained as pellets from Dow Chemical. A 12.5% (w/w) solution made from pellets directly dissolved in dimethyl formamide (DMF, Merck, pro analysis) was designated PEL. Extraction of Pellethane was performed with ethanol in a Soxhlet extractor. The pellets were extracted for 72 h in refluxing ethanol and dried for 72 h at 40 °C before dissolving in DMF to a 12.5% (w/w) solution. This solution was designated PEL-EX. Both solutions were filtered under N_2 purge at 5 °C through a 5 μm TEFLON filter (Millipore) for elimination of gel particles.

Two solutions of a poly(ether urethaneurea) (20% w/w), PUUR and PUUR-A, prepared from PTMG 2000, MDI and ethylene diamine (EDA) in dimethyl acetamide were gifts from E.I. Du Pont de Nemours & Co., Inc. The molecular composition of PUUR-A was similar to that of Biomer [21]. PUUR had the same composition as PUUR-A except for 5% (w/w) of Methacrol 2138F additive, i.e. poly(2-diisopropylaminoethyl methacrylate-co-decyl methacrylate) in PUUR-A [19].

2.2. XPS analysis and contact angle measurements

The procedures have been described previously in detail [22]. The PUUR and PUUR-A solutions were diluted to 10% before use. For the XPS analysis films were prepared by casting two layers of polymer on clean glass dishes with drying for 24 h at 60 °C after each casting. Final drying was done at 60 °C for 24 h in a vacuum oven.

X-ray photoelectronic spectra (XPS): The chemical composition of the surfaces were analysed with an XPS-spectrometer (AEI ES200B). Excitation X-ray source was AlK_α (1486.6 eV) and the operation condition was set at 14 kV and 20 mA. High resolution spectra of C1s, O1s and N1s were obtained with a scan width of 25 V and a scan rate of 0.02 $V s^{-1}$ except for nitrogen, which was set at 0.01 $V s^{-1}$. The different band areas were normalized by using Scofield section factors, C1s = 1.00, O1s = 2.93 and N1s = 1.8 [23]. Measurements were made on the air-facing side of the films.

Contact angle measurements: Films used for contact angle measurements were cast (single layer) on ultra-clean glass plates and dried at 60 °C for 24 h followed by final drying at 60 °C for 24 h in vacuum. The dry films displayed a glossy surface and showed no roughness on the micro-scale as measured by scanning electron microscopy (SEM). Photographs were taken of drops of doubly distilled water placed on the polymer surfaces by a motor driven syringe. Advancing and receding contact angles were determined by increasing and decreasing the size of the drops, and the angles were evaluated from photographs. Values for the contact angles given in Table I are averages of seven measurements, with a standard deviation of 1–3°.

2.3. Surface preparations for protein adsorption and ellipsometric experiments

The polymers were deposited onto 0.2 mm thick, polished silicon wafers from Wacker Chemie, Münich, Germany. The silicon wafers were washed in a 1:1:5 solution of NH_4OH (25%), H_2O_2 (30%) and H_2O at 80 °C for 5 min and rinsed in deionized water. They were then washed in a 1:1:6 solution of HCl (37%), H_2O_2 (30%) and H_2O at 80 °C for 5 min and rinsed in deionized water. This treatment gives a clean, hydrophilic surface. The cleaned surfaces were dried in flowing N_2 followed by heating in an oven at 60 °C for 20 min. They were then transferred into 1% (w/w) polymer dissolved in dimethylformamide. After 1 min of deposition time, the surfaces were dried in air at 50 °C for 20 min.

The thicknesses of the polymer layers were measured in air with an automatic AutoEll 2 Rudolph Research ellipsometer, N.Y., U.S.A. (λ = 632.8 nm, angle of incidence 70°), and varied from 20 nm to 70 nm. The adsorbed amount of organic material was calculated from ellipsometric data according to the method described by Elwing *et al.* [24]. Prior to plasma incubations the samples were stored in 0.01 M phosphate buffered saline (PBS) with sodium azide (0.02%), pH 7.4, for 18 h or longer. Light microscopic examination of the PBS-stored surfaces indicated no dissolution or peeling of the polymer film, although ellipsometric data did indicate some swelling. The surfaces were transferred into a glass cuvette filled with the PBS (see Fig. 1) and the film thickness was measured. The buffer was pumped out and citrated 2 ml plasma, diluted to 10% in the PBS, was immediately injected into the cuvette (volume 2 ml). After 10 min of incubation at room conditions, the plasma was pumped out and the cuvette was rinsed five times using the PBS. This was followed by antisera and

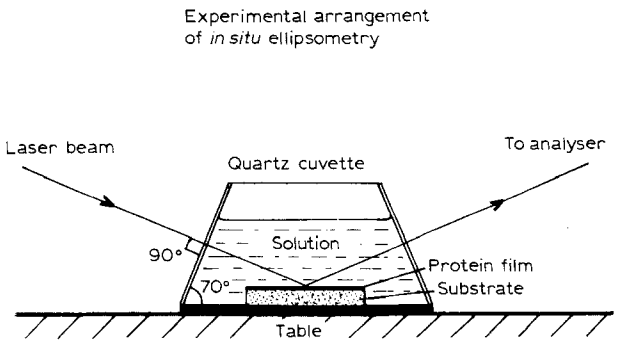

Figure 1 Schematic drawing of the experimental arrangement of *in situ* ellipsometry.

amplification incubations, 15 min each as described below. Finally the cuvette was rinsed five times in PBS. After each incubation and rinsing sequence, the amount of adsorbed organic material was measured *in situ* using the two-zone ellipsometer algorithm in buffer at the same spot for all polymer, protein adsorption and antisera incubation steps of a single sample. Throughout the procedure, the refractive index $n = 1.465$ was assumed for both the polymeric and adsorbed organic material. The assumed refractive index for the buffer solution was 1.333.

2.4. Blood and antiserum

Phosphate buffered saline (PBS), pH 7.4 was used for human blood plasma dilution and rinsings. All protein experiments were performed in triplicate, and different plasma and surface cleaning batches were used for different experimental series. The results obtained were consistent both for whole and diluted plasmas.

Citrated blood from one apparently healthy blood donor was centrifuged at 1500 g for 20 min and the collected plasma was stored at −80 °C until use. SwαAlb and SwαIgG were obtained from Orion Diagnostica, Finland, RbαFG, RbαC3c and RbαFN from Dakopatts, Denmark, and GAαHMWK, GAαFXII, GAαLP (a and b chains), GAαFVIII, and GAαAThIII were purchased from Nordic Immunochemical Laboratories in the Netherlands. All antisera were polyclonal and were used without further purification. Amplifications were done using RbαGA IgG from Dakopatts and SwαRb IgG from Orion. Control tests showed small antisera cross-reactivities and amplification antisera had low affinity for surfaces incubated in human plasma. Human IgG (1 mg ml^{-1}) was added to the amplification antisera in order to further suppress any residual cross reactivity with surface located proteins. Anti-Alb, α-IgG, α-FN, α-C3c and α-FG were diluted 1/20, and the other antisera, 1/50 in PBS.

All quantification of proteins referred to in this work are representative values and not statistical means ($n = 3$). The measured maximum deviations were +/− 20%.

3. Results

The surface characterization by XPS and contact angle measurements shows that the polymer additives

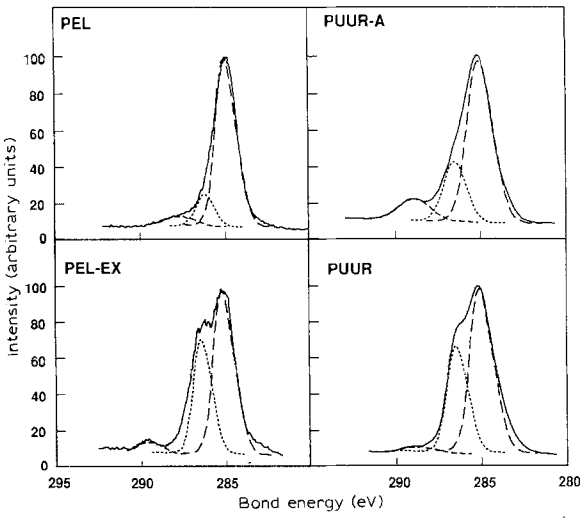

Figure 2 XPS surface analysis of the polymers: carbon peaks.

largely determine the composition of the surfaces. Extraction of Pellethane with ethanol removed most of the processing aids and low molecular weight oligomers. The contact angles were found to be significantly lower for the PEL-EX surfaces than for PEL surfaces, i.e. the surfaces became more hydrophilic (Table I). The surface composition, as determined by XPS, was also changed by extraction (see Fig. 2). Due to the presence of the fatty acid amide processing aid, the aliphatic carbon peak (285 eV) dominated the spectrum of PEL. This was also reflected in the large C/O ratio (Table II). PEL-EX, on the other hand, showed increased relative amounts of ether carbons (C-O-C, 286.5 eV) and carbonyl (carbamate 289.3 eV) carbons in the surface, as would be expected from a poly(ether urethane) based on PTMG (see Fig. 2).

For the poly(ether urethaneurea)s PUUR and PUUR-A, the surface compositions were quite different due to the presence of the polyacrylic additive, poly(2-diisopropylaminoethyl methacrylate-co-decyl methacrylate). Wu *et al.* [25] and Tyler *et al.* [26] have previously reported on the influence of additives for surface properties of PUUR and Biomer. Changes in wetting behaviour as well as mechanical properties of these polymers are due to the Methacrol additive, and were investigated by Freij-Larsson *et al.* [27]. Because of the accumulation of the acrylate at the surface

TABLE I Water contact angles (values reported are mean values of seven measurements, with a standard deviation less than 3°)

Polymer	θ_{adv}	θ_{rec}	Hysteresis
PEL-EX	77	56	21
PEL	84	68	16
PUUR	80	60	20
PUUR-A	90	28	63

TABLE II Elemental ratios on polyurethane surfaces from XPS analysis

Surface	C/O	C/N
PEL-EX	6.2	32
PEL	10.5	33
PUUR	6.4	34
PUUR-A	5.5	48

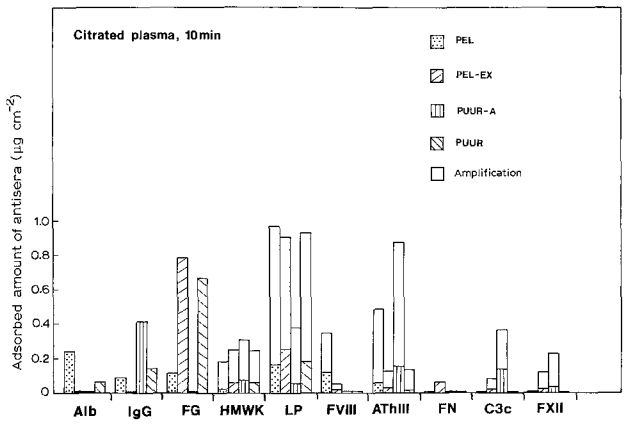

Figure 3 Antisera binding patterns for the various polyurethanes. In order to visualize low abundant plasma proteins, they were amplified by addition of anti-antibodies (e.g. RbαGA) onto the primary antibodies (GAαHMWK).

the XPS-spectra of PUUR-A displayed increased amounts of carbonyl carbons at 288.3 eV and aliphatic carbons, and a decrease in ether carbons as compared to PUUR. Due to the presence of strongly hydrophobic and hydrophilic groups, the polymer is amphiphilic, introducing a large contact angle hysteresis as compared to the original PUUR surface. The exact ratio between the co-monomers of the Methacrol additive is not known. However, a 70/30 ratio of isopropylaminoethyl methacrylate to decyl methacrylate was assumed by Belisle [19]. The high C/N ratio found for the PUUR-A surface (Table II) thus indicates that under the conditions used in the XPS analysis the hydrophobic decyl groups are more exposed at the surface than the more hydrophilic tertiary amine groups. These results are in accordance with data presented by Tyler *et al.* [26].

After immersions in human plasma the polymer surfaces were tested for antisera binding and quantified *in situ* by the use of ellipsometry (see Fig. 3). On the extracted Pellethane significant amounts of α-FG and α-LP were detected. Small amounts of α-HMWK, α-FN, α-ATh III, α-F XII, α-IgG and α-C3c were also found. However, the unextracted Pellethane, containing the processing aids, showed a different antisera binding pattern. On this polymer small amounts of α-Alb, α-IgG, α-FG, α-HMWK, α-LP, α-F VIII, and α-ATh were measured. The most significant differences in the extracted Pellethane were increased α-Alb and α-lgG and decreased α-FG depositions, respectively.

The poly(ether urethaneurea) containing the acrylic polymeric additive, PUUR-A, did not bind α-FG, but increased quantities of α-IgG, α-ATh III, and α-C3c. Small but significant depositions of α-HMWK, α-LP and α-F XII were also found. The pure extracted polymer (PUUR), however, adsorbed increased quantities of α-FG and α-LP and the adsorption of α-IgG and α-C3c were decreased as compared with PUUR-A.

4. Discussion

4.1. General

The antisera method used to determine specific adsorbed organic material is only semi-quantitative. The measured values depend on the correctness of the ellipsometric film model, the choice of adsorbed protein refractive index, the antisera concentration, antigen–antibody affinities, and on the number of protein epitopes available. In this study, the antisera concentrations were high in order to avoid diffusion limitations and to obtain short incubation times, thus minimizing influence of protein desorption and surface proteolytic activity. Unspecific binding of antibodies, on the other hand, increases with increased antisera concentration, but was not observed as a problem in this work. Since an amplification antisera was used for some antisera detections the results in Fig. 3 should be compared only for each protein and do not reflect quantitative comparisons between the different proteins on a specific surface. In spite of the uncertainties mentioned above, relative comparisons, as made in the present study, are valuable for probing differences in surface biological activity.

For both types of base polymers, the additives used significantly decreased the α-FG deposition after the plasma immersions. This would suggest a low fibrinogen mediated cell binding on to the PUUR-A and PEL surfaces in contact with blood. Furthermore, all four polymers displayed low α-HMWK, α-F XII, and α-FN depositions indicating a low intrinsic pathway activation. The low tendency for deposition of the proteins as mentioned above, and possibly other proteins, may serve as an indicator for blood compatible materials in general. But to confirm this correlation additional experiments are necessary.

The polymers did, however, show significant individual variations. PEL had an increased binding of α-Alb and α-F VIII. This is interesting since albumin is sometimes immobilized onto surfaces in order to improve their blood compatibility. Furthermore, the low α-C3c deposition onto PEL indicates a low complement activation. The hydrophobic PEL surface is, according to the XPS spectra, mostly composed of alkyl groups. This preference for albumin binding onto surfaces containing long-chain alkyl groups has previously been reported by Grasel *et al.* [6] and Munro *et al.* [7, 8].

The wettability properties of PEL were changed upon extraction (PEL-EX) as seen in Table I, and the extraction polymer showed increased α-FG binding. This suggests a fibrinogen-mediated cell deposition *in vivo* and may provide a justification for using the additive as means of improving the haemocompatibility of the polymer.

The experiments with PUUR-A showed the lowest α-FG, and significant α-IgG and α-ATh III depositions. The results also suggest a possible complement activation as indicated by the increased α-C3c and α-IgG depositions. The interpretation of the latter finding is however somewhat unclear at present time. The PUUR antisera adsorption pattern was similar to that of PEL-EX except for the slightly increased binding of α-IgG onto the PUUR surface.

The low α-FG and α-HMWK binding for PEL and PUUR-A noted in the present study is interesting, and the polymer properties giving rise to this should be compared with other data on properties and morphology of segmented polyurethanes, e.g. by Wang and

Cooper [28]. Preliminary *ex vivo* blood coagulation and platelet deposition tests using PUUR and PUUR-A surfaces have also indicated a higher thromboresistance for PUUR-A, i.e. the polymer containing the Methacrol additive in this study [27].

Polymeric additives may be used to modify the hydrophilic properties of polymer surfaces [22]. The Methacrol additive is incompatible with the poly(ether ureaurethane) used in the present investigation and influences the morphology of the base polymer [25, 27]. Dynamic mechanical measurements indicated that the presence of the acrylic polymer gave rise to a more phase separated structure [28, 29]. Furthermore, increased amounts of aliphatic carbons and carbonyl groups were observed in the XPS spectra together with an increased C/N ratio (see Table II) [cf. 26]. The large contact angle hysteresis noted for the PUUR-A surfaces can be explained by a phase separated surface morphology displaying hydrophilic and hydrophobic domains. An alternate explanation is that the additive forms an amphiphilic layer on the surface.

Contact angle measurements indicate that the pure polymers PUUR and PEL-EX possess similar surface properties, although it may be assumed that the PUUR surface is more separated into microphases. The urea groups of PUUR are considered to be hydrogen bonded to a greater extent than the urethane groups of PEL-EX giving rise to more well-defined semi-crystalline domains [29].

4.2. Comparison with surface antisera adsorption patterns on solid surfaces

We have obtained antisera binding patterns which may be compared with those of a well known coagulation intrinsic pathway activator, solid hydrophilic silica. Hydrophilic silicon surfaces incubated in human plasma bound no α-FG, α-ATh III or α-C3c, although increased amounts of α-HMWK, and significant quantities of α-F XII and α-PK were found [30]. When the silica surfaces were rendered hydrophobic ($\theta \approx 60$–$90°$) by methylation, high amounts of α-FG and low quantities of the other antisera were deposited [30]. Interestingly, methylated silica with $50° < \theta < 70°$ (mixed polar/hydrophobic character) adsorbed low amounts of all tested antisera and displayed the lowest total adsorption of plasma [30]. PEL-EX and PUUR in the present work show antisera binding patterns similar to those for hydrophobiced silica ($\theta > 70°$), while the (amphiphilic) PUUR-A resembles methylated silica with mixed polar/hydrophobic properties ($50° < \theta < 70°$).

The present results (assuming lowered deposition of fibrinogen is indicative of thromboresistant materials) agree well with the hypothesis that mixed polar/non-polar surfaces have increased thromboresistance, as suggested by Ratner *et al.* [9] and Merrill [29]. The present and previous studies have not, however, separated interactions related to the surface free energy from those originating from specific chemical or steric interactions caused by different functional groups and variations in chain conformations. For these studies various surfaces with immobilized molecules (e.g. alkane-thiols on gold [31, 32]) would be more appropriate.

5. Conclusions

The present study deals with well known biomedical polymers and their antisera binding patterns after exposure to human plasma. The relatively blood compatible surfaces of Pellethane and the Biomer-like polyurethane both showed simultaneous low α-HMWK, α-F XII, α-C3c, and α-FG depositions. We suggest antisera binding patterns similar to those obtained in this work are a good first approximation for blood compatible surfaces. Antisera and ellipsometric methods thus offer a quick tool for assessing surface biological activity.

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