

Surface modification of a segmented polyetherurethane using a low-powered gas plasma and its influence on the activation of the coagulation system

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Abstract

A medical grade segmented polyetherurethane (PEU) was treated with a low-powered gas plasma using O₂, Ar, N₂ and NH₃ as the treatment gases. Changes in the surface functional group chemistry were studied using X-ray photoelectron spectroscopy. The wettability of the surfaces was examined using dynamic contact angle measurements and the surface morphology was evaluated using atomic force microscopy. The influence of the surface modification to the polyurethane on the blood response to the polyetherurethane was investigated by measuring changes in the activation of the contact phase activation of the intrinsic coagulation cascade. The data demonstrate that the plasma treatment process caused surface modifications to the PEU that in all cases increased the polar nature of the surfaces. O₂ and Ar plasmas resulted in the incorporation of oxygen-containing groups that remained present following storage in an aqueous environment. N₂ and NH₃ plasmas resulted in the incorporation of nitrogen-containing groups but these were replaced with oxygen-containing groups following storage in the aqueous environment. In all plasma treatments there was a lowering of contact phase activation compared to the untreated surface, the N₂ and NH₃ treatments dramatically so.

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1. Introduction

Polyurethanes (PUs) have been widely used in the medical device industry because of their good mechanical and biological properties, i.e. high tensile strength, lubricity and biocompatibility [1]. It is well understood that polyester-urea-urethanes are susceptible to hydrolytic degradation [2,3] and polyether urethanes to oxidation [4,5] and consequently some concerns have been raised about the feasibility of their use in long-term biological environments. However, the use of PUs in short- and medium-term applications is widely accepted, whilst their intrinsic biostability and therefore potential

for long-term application is being continuously improved [6,7].

PUs, as a general class of materials have blood-contacting properties that are suitable for short-term applications such as vascular cannulae [8,9]. Use of PU in longer-term applications such as indwelling central venous access devices presents problems of a thrombotic nature [10]. In addition to thrombophlebitis [11,12], which is related to material hardness and vessel wall irritation, reported problems due to the use of indwelling catheters are characterised by surface thrombus [13–15], which can reduce the performance of catheter-mounted biosensors [16], embolism [15,17] and vessel occlusion [18], which are clearly undesirable.

It is accepted that the host response to the insertion of a biomaterial into blood is controlled by the nature of the proteins adsorbed on the surface and their conformation, which is governed by the chemistry and energetics of the surface. Other than the influence of

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negative charge [19], fatty acids [20], various crystalline substances [21] and some other generic structures, it is not yet clear *how* these different parameters control the degree of initial factor XII (fXII) activation, and therefore the initiation of the intrinsic coagulation cascade. Ultimately, contact activation, whereby fXII and its cofactors are assembled in such a way that it is activated to become fXIIa, and then able to activate fXI, can proceed only when the surface chemistry allows a suitable conformational arrangement of adsorbed fXII that allows autoactivation. The rate of the progression of cascade activation, therefore, is dependant on the specific spatial arrangement of chemical species and their number at the surface. The arrangement which either permits or prevents correct conformational organisation is as yet unknown.

Many different formulations of PU are available (different soft segment chemistries, e.g. ethers, esters and carbonates; backbones based on aliphatic or aromatic structures), presumably with different surface chemistries. However, experience of their use in biological environments has shown that PUs tend not to exhibit greatly different responses to these environments. Surface modifications of PU using coating technologies or macromolecular attachment have been investigated. For example, use of hydrophilic coatings such as polyvinylpyrrolidone (PVP) have been utilised on intravascular devices with some success [22] as a strategy for improving thromboresistance, but the manufacturing process is made much more complex with the potential for complicated regulatory approval issues. Likewise, surface modification with anticoagulants [23,24] and other macromolecules [25,26] has been utilised over many years but is complicated and expensive. Use of certain dialysis membranes and other high surface area: volume ratio applications has highlighted the potential problems resulting from the activation of the contact phase of the intrinsic coagulation pathway despite effective coagulative therapy [27]. The activation of the contact phase system is known to result in the release of vasoactive peptides such as bradykinin from high molecular weight kininogen [28,29], which can lead to highly dangerous systemic conditions such as anaphylactic shock. Heparin is well known for its effective anticoagulant properties [30] and has contributed to the success of invasive cardiovascular procedures [31]. On the other hand, heparin, including its low molecular weight fractions, have been implicated in the enhanced aggregatory response of platelets in high shear environments [32,33]. Many manufacturers would find it desirable, therefore, to be able to modify the performance of PUs without expensive manufacturing processes. Modification of the surface chemistry whilst preserving the bulk chemical properties would be highly attractive. Preparing surfaces which have the added advantage of reducing undesirable biological activation

which would otherwise be masked by attached anti-coagulants would be additionally beneficial.

Gas plasma modification has been used previously as a strategy for enhancing various polymer properties [34], for example enriching the surface with oxygen species [35,36], nitrogen species [37,38] or more complex functional groups [39]. Most chemical modifications have only a limited effect on the biological response to polymers and it has not been shown previously that the activation of the blood coagulation system can be extensively reduced by gas plasma modification.

The aim of the present study was to change the surface properties of a commercial PU without the need to introduce additives or chemical groups, which could leach out of or migrate to the surface in an uncontrolled fashion. If the nature of the surface can be controlled such that it is relatively stable to hydrolytic and oxidative processes [40], while at the same time improving cellular interactions, a new generation of polymers with enhanced biocompatibility and biostability may be possible.

In this study the surface of a medical grade polyetherurethane (PEU) was modified by treatment in a low-powered gas plasma. The modifications thus produced were analysed using X-ray photoelectron spectroscopy (XPS), dynamic contact angle measurements (DCA) and atomic force microscopy (AFM) immediately after treatment and following aging in air or an aqueous environment. The influence of the surface modifications on the thrombogenicity of the surfaces was assessed by measuring the activation of the contact phase of intrinsic coagulation using a partial thromboplastin time (PTT) assay.

2. Materials and methods

2.1. Materials

An additive-free thermoplastic medical grade PEU was obtained from Eurothane Ltd (Worcester, UK) in 2 mm thick sheets. The PEU comprised alternating “hard” and “soft” segments of 4,4-diphenyl-methyl diisocyanate (MDI) and polytetramethylene oxide (PTMO), respectively, with butanediol as the chain extender. The polymer was cut into 10 × 20 mm pieces and cleaned using diethyl ether to remove surface contaminants and low molecular weight species. Thereafter all samples were handled with surgical gloves and tweezers to minimise contamination.

Four ultrapurity grade reactor gases, O₂: 99.5%, Ar: 99.998%, N₂: 99.998% and NH₃: 99.99%, (BOC, Guildford, Surrey, UK) were used as supplied. Two liquids were used in contact angle measurements, phosphate-buffered saline solution (PBS from Sigma, Poole, UK) and 1-bromonaphthalene (purchased as a

98% liquid from Aldrich, Poole, UK). PBS was made up in distilled water following the manufacturer's instructions. This yielded a solution of pH 7.4 with the following composition: 10 mM phosphate buffer (80% NaH_2PO_4 and 20% Na_2HPO_4), 270 μM KCl and 137 mM NaCl.

2.2. Plasma treatment

The radio frequency (rf) equipment and treatment procedure used in this study has been outlined in detail previously [37]. Briefly, a low-powered plasma was produced in a half-wave helical resonator formed from a 100-turn copper wire wound directly on the outside of a glass tube. The excitation frequency and rf power being 13.6 MHz and <1 W, respectively. In all experiments, treatment time was fixed at 1 min, the gas pressure set at 8×10^{-2} mbar and the flow rate 85 sccm. The gas species admitted to the reactor (O_2 , Ar, N_2 and NH_3) was the only variable implemented during sample treatment.

2.3. Ageing procedure

Materials were stored in plastic vials containing air or PBS at 37°C in the dark for time periods of up to 1 month. Untreated specimens underwent the same ageing procedure for comparison. Samples aged in air were wrapped in aluminium foil to minimise hydrocarbon contamination, whereas samples aged in PBS were thoroughly washed in deionised water and then dried using pressurised air before analysis.

2.4. X-ray photoelectron spectroscopy (XPS)

Detailed characterisation of the untreated and modified surfaces was carried out using XPS to evaluate the elemental and chemical groups present in the surface. A VG Scientific ESCALAB MXII spectrometer (East Grinstead, West Sussex, UK) with a 260 W AlK_α radiation source was used to obtain spectra in CAE (constant angle energy) mode. The operating pressure was maintained at 10^{-8} mbar enabling the field emission gun to deliver a primary beam current of 10 nA onto the sample as a 30 nm spot. A pass energy of 100 eV was used to record the widescan spectra (0–1000 eV) and 20 eV for high-resolution C1s, N1s and O1s spectra. All measurements were obtained at a take-off angle of 45°, corresponding to an approximate depth profile of 5 nm. Binding energies were referenced to the C1s hydrocarbon peak set at 285 eV, with FWHM of 1.6 eV, such that surface elemental compositions could be calculated from the relative peak areas. The C1s, N1s and O1s envelopes were analysed and peak-fitted using a combination of Gaussian and Lorentzian peak shapes obtained from the ESCALAB software package.

2.5. Dynamic contact angle (DCA)

DCA measurements were obtained using the Wilhelmy plate technique [41] to determine the hydrophilicity of the PEU surface and the effect of plasma treatment on surface wettability. A number of advancing (θ_A) and receding angles (θ_R) were recorded using a Cahn DCA322 surface force analyser (Manchester, UK) to allow for standard errors. Treated and untreated surfaces were consecutively immersed in and removed from PBS and 1-bromonaphthalene at a speed of 60 $\mu\text{m min}^{-1}$. Curves relating the interfacial tension to the immersion depth were plotted and used to calculate θ_A and θ_R , the advancing and receding contact angles, respectively. A representative contact angle was calculated for each formulation using the mean and standard deviation of six independent measurements.

2.6. Atomic force microscopy (AFM)

The polymer surfaces were examined on a sub-micron scale using AFM [42,43] to evaluate the effect of the surface treatment on the surface morphology. A Digital Instruments Nanoscope III AFM (Santa Barbara, CA, USA) equipped with a 16 μm scanner and a multimode AFM head which could be operated in both "contact" and "tapping" mode to record images was employed. The latter mode was chosen since it has the advantage of offering low shear and contact forces and a 125 nm etched silicon tip resonant at 300 kHz was used. Tests were repeated over approximately four sample areas to improve the reproducibility of the results.

2.7. Contact phase activation

Contact phase activation of the intrinsic coagulation cascade was estimated by way of a PTT assay [44]. Venous blood was collected from consenting adult male volunteers who had fasted for more than 4 h and had been free from medication for at least 14 days. Phlebotomy of a median cubital vein was performed using a 19-G needle without the use of a tourniquet to reduce the release of tissue factor. The blood was anticoagulated by adding a one-tenth volume of 3.8% (w/v) tri-sodium citrate. Platelet poor plasma (PPP) was produced by centrifugation of blood aliquots at 13 000 g in a microcentrifuge. A large pool of unactivated PPP with little variation in coagulation activation was created by collecting together the plasmas of several volunteers, then snap freezing individual 250 μl aliquots in 500 μl eppendorf tubes in liquid nitrogen. To perform plasma-surface contact, aliquots of PPP were defrosted and pre-warmed for exactly 1 min at 37°C in a water bath. PTT assays were performed by statically incubating surfaces with 200 μl of PPP at 37°C for 10 min in a humidified chamber. Following incubation, 80 μl of PPP

were removed from the surface and added to 80 μl of 25 mM CaCl_2 and 80 μl platelet substitute (partial thromboplastin) (Diagnostic Reagents Ltd, Thame, UK), both already pre-warmed to 37°C. The clotting time of the plasma after the addition of CaCl_2 was measured by following the turbidity of the plasma using an Instrumentation Laboratory ACL300 Research coagulometer (Milan, Italy). Experimental variation (e.g. time, temperature) was reduced by performing the incubations and coagulation determinations for all materials at the same time. Plasma turbidity of each sample was determined 10 times per second for 400 s.

Table 1
Effect of ageing environment on the surface elemental ratios of the PEU modification

Treatment	Elemental ratios		
	O/C	N/C	N/O
Untreated	0.18(0.01)	0.05(0.01)	0.32(0.01)
1 month in PBS	0.19(0.01)	0.07(0.01)	0.37(0.01)
O_2 treated	0.29(0.01)	0.04(0.01)	0.14(0.01)
1 month in PBS	0.26(0.01)	0.05(0.01)	0.14(0.01)
1 month in air	0.15(0.01)	0.05(0.01)	0.36(0.01)
Ar treated	0.23(0.01)	0.03(0.01)	0.13(0.01)
1 month in PBS	0.28(0.02)	0.05(0.01)	0.13(0.01)
1 month in air	0.20(0.02)	0.03(0.01)	0.15(0.01)
N_2 treated	0.18(0.01)	0.24(0.04)	1.33(0.04)
1 month in PBS	0.24(0.01)	0.06(0.01)	0.25(0.01)
1 month in air	0.26(0.01)	0.11(0.01)	0.42(0.01)
NH_3 treated	0.24(0.01)	0.18(0.01)	0.75(0.01)
1 month in PBS	0.30(0.01)	0.04(0.02)	0.20(0.01)
1 month in air	0.20(0.01)	0.06(0.01)	0.30(0.01)

The atomic ratios are determined from the relative areas of the C1s, O1s and N1s peaks for the virgin and modified PEU material. Presented as the mean and standard deviation in brackets for $n = 3$.

Table 2
Representative XPS high-resolution C1s peak fit data for untreated, plasma treated and aged PEU surfaces

Treatment	-C-R-	-C-N-	-C-O-C-	-C=N-	-C=O- or -N-C-O-	-HNCOO-
Untreated	56.5	4.3	34.8			4.3
1 month in PBS	58.7	4.4	31.5			4.4
O_2 treated	47.6	3.5	36.9		8.3	3.7
1 month in PBS	47.4	3.7	37.1		7.8	4.0
1 month in air	63.6	3.6	25.6		5.0	2.1
Ar treated	44.4	3.4	38.2		10.0	3.8
1 month in PBS	41.4	3.7	36.1		13.1	5.6
1 month in air	52.8	3.5	32.6		7.4	3.7
N_2 treated	37.7	5.7	27.8	12.9	10.8	5.0
1 month in PBS	59.5	3.8	22.0	6.0	5.7	2.9
1 month in air	52.2	4.8	23.7	10.0	6.6	2.8
NH_3 treat	41.0	5.0	30.0	12.0	8.0	4.0
1 month in PBS	37.3	4.8	30.0	10.3	13.0	4.5
1 month in air	40.3	5.7	30.9	9.3	10.0	3.9

The relative percentages of each component were recorded after Gaussian and Lorentzian peak fitting algorithms were applied to the data.

Unactivated PPP and kaolin-treated plasma (500 $\mu\text{g}/\text{ml}$ kaolin final concentration) were used as the negative and positive controls, respectively.

3. Results

3.1. XPS

Widescan and high-resolution XPS spectra were recorded for the PEU material to determine the existing functionality present in the surface region and show subsequent changes in species introduced by each treatment. Table 1 shows the corrected atomic ratios determined from the relative areas of the C1, O1 and N1s peaks for the virgin and modified PEU material as means and standard deviations in brackets for three samples. Peak fitting of the C1s spectral envelope is commonly employed in XPS to estimate the proportion of different carbon bonds at the surface of a polymer [34,45] and Table 2 summarises the relative percentages of each component observed after Gaussian and Lorentzian peak fitting algorithms are applied to the data. The data presented are representative of the changes measured.

The C1s peak for the untreated PEU surface can be resolved into four component peaks at 285, 285.8, 286.5 and 289 eV (Fig. 1a). These peaks correspond to aliphatic carbon [C-R, where R denotes C or H], amine [C-N], ether [C-O-C] and carbamate [HNCOO], respectively. The latter two oxidative functionalities are an integral part of the repeat unit for soft and hard segments, respectively.

Following oxygen plasma treatment there is an increase in the intensity of the O1s signal relative to that of the C1s. This is concomitant with a decrease in the N/O ratio. Consequently, the shape and intensity of

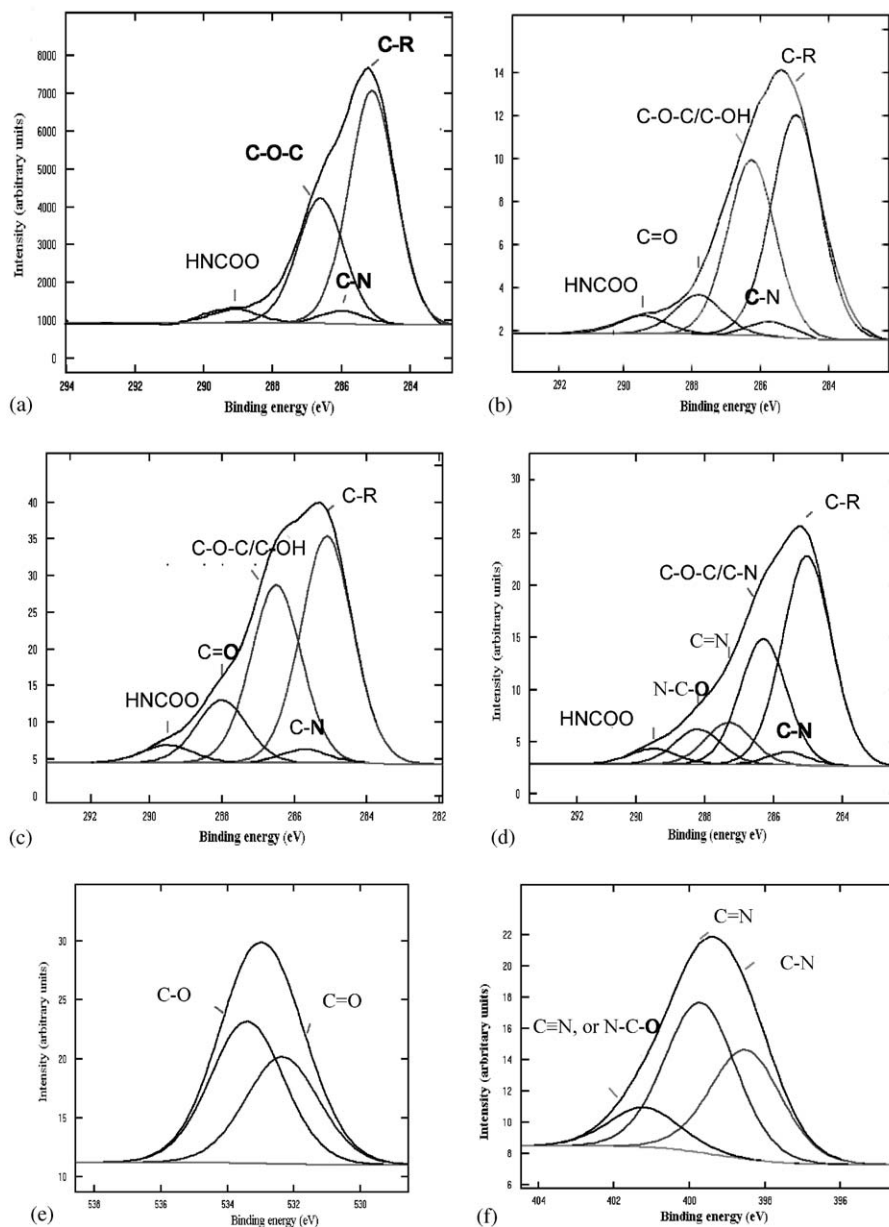


Fig. 1. High-resolution XPS spectra of the C1s peak for (a) untreated; (b) O₂ treated; (c) Ar treated; (d) N₂ treated PEU; (e) O1s peak for Ar treated PEU; (f) N1s peak for N₂ treated PEU.

the C1s spectrum is affected such that the envelope is broadened and can be deconvoluted into five peaks as shown in Fig. 1b. In addition to the peaks identified for the untreated material, there is also a further peak at 288 eV that corresponds to a carbon experiencing greater electron delocalisation than that of the C–O–C ether environment. This can be attributed to some kind of C–O_x functionality and probably results from O–C–O or C=O. It is also likely that the increase in the component identified at 286.5 eV could infer C–OH bonds [46] but binding energies are indistinguishable from the ether functionality and cannot be separated.

The susceptibility of this polymer to Ar plasma can be illustrated by the decrease of the C1s and N1s with respect to the O1s signal, resulting from the incorporation of oxygen-containing groups. Changes to the symmetry of the C1s envelope, Fig. 1c, are very similar to those observed for the oxygen plasma including the appearance of the component at 288 eV. Changes in the O1s envelope (Fig. 1e) also fit this explanation. Since the nitrogen-containing functionality is only present in the hard urethane blocks of the PEU [47] the small reduction of the N/C ratio after treatment with O₂ and Ar plasmas could represent changes in the concentration of hard segment at the surface region

compared with the untreated material. Alternatively, this suggests surface enrichment with polyether soft segments, which is consistent with the increase of the peak at 286.5 eV in the C1s high-resolution spectra.

In the case of nitrogen plasma treated PEU there is significant incorporation of nitrogen-containing groups. The O1s signal is unaffected, but it is likely that the incorporation of oxygen moieties occurs on exposure to air after plasma treatment but at the expense of oxygenated molecules lost at the surface during plasma exposure. The exact identification of functionalities bound to the surface is difficult, however the C1s spectrum can be deconvoluted into six peaks as illustrated in Fig. 1d. In addition to C–O–C type moieties the component at 286.5 eV may include C–NH₂, C–NH or C–N=C groups. The peak located at 287 eV corresponds to C=N, whilst that at 288 eV could imply N–C–O, CONH₂ or O–C–O type functionalities. However, on the basis of the amount of nitrogen incorporated compared to that of oxygen, it is likely that nitrogen-containing species have formed (Fig. 1f). The surface chemistry of the NH₃ plasma treated surface was very similar to the N₂ treated surface resulting in the incorporation of nitrogen-containing groups.

The effect of ageing for 1 month in PBS on the surface chemistry of the untreated material is negligible and the C1s spectra shows only a small enhancement associated with the carbamate component identified at 289 eV. Following storage in PBS of the oxygen plasma treated samples there is little effect on the intensity of the O1s and N1s signals and the full-width at half-maximum (FWHM) of the C1s is comparable with that obtained for immediate plasma processing. There is, however, slight enhancement in the 286.5 eV region of the spectrum (Table 2) and this can be attributed to additional incorporation of oxygen-containing species, i.e. C–O–C/C–OH bonds. This signifies that the changes occurring in the surface region following O₂ plasma treatment are relatively stable in the aqueous medium. The Ar plasma treated surface shows a relative increase in the O1s peak with respect to the C1s signal after 1 month in PBS such that the C1s envelope is very similar in shape and has the same characteristics when deconvoluted as that of the O₂ plasma treated surface. In the case of N₂ plasma treatment, there is a relative reduction in intensity of the N1s, concomitant with an enhancement of the O1s signal following 1 month storage in PBS. There is a reduction of the FWHM of the C1s envelope which appears to be more symmetrical and less pronounced at the higher binding energy region of the spectrum. This suggests that storage in an aqueous environment facilitates loss of N₂ containing species introduced by this plasma and replacement with hydroxyl groups. As above, similar results were observed following PBS storage of the NH₃ treated surface.

Storage in air had an effect on the chemistry of O₂ treated surfaces in particular. There was a considerable decrease in intensity of the O1s signal compared to the relative intensities of the C1s and N1s peaks and this can be clearly seen by the elemental ratios in Table 1. There was also a decrease in the FWHM of the C1s envelope and the peak heights of the oxygen-containing functionalities at 286.5 and 288 eV are most affected in intensity (Table 2). The Ar plasma treated sample also shows the same trends in the C1s envelope as that for O₂ treated PEU, but to a lesser extent. This would imply that the Ar plasma causes changes in the surface structure which are not affected in the same way as for O₂ plasma processing and induces a relative degree of permanency within the surface region. There is very little difference in the FWHM for the C1s envelope obtained for the N₂ plasma treated surface after ageing in air compared with that following storage in PBS, except a slight increase in the N-containing groups suggesting some kind of chain reorientation where the surface free energy is favoured by the hard segment.

3.2. Contact angle measurements

The rationale behind recording wettability measurements is that the technique provides information about the interfacial region of a biological medium and has a high degree of sensitivity regarding the very uppermost layers of the PEU/air interface. In such a way it is possible to correlate the findings with molecular information collected by XPS. This creates a more accurate picture of the functionality dominating the polymer interfacial region. Changes to the polar and apolar components of the total surface energies, of untreated and plasma treated materials were obtained by measuring contact angle in PBS (θ^{pbs}) and 1-bromonaphthalene (θ^{bro}). Untreated surfaces exhibited both hydrophobic and hydrophilic behaviour characterised by relatively high values of advancing angle ($\theta_{\text{A}}^{\text{pbs}} = 85^\circ$) and low receding angles ($\theta_{\text{R}}^{\text{pbs}} = 42^\circ$). All plasma treatments caused a significant reduction (Table 3) in the $\theta_{\text{A}}^{\text{pbs}}$ to values in the range 46–62° as shown in Fig. 2a. The effect of plasma treatment on $\theta_{\text{R}}^{\text{pbs}}$ was not significant for any surface treated except NH₃ ($p = 0.0013$) and occurred in the range 29–43°. The contact angle hysteresis, $\Delta\theta$ was found to be small, in the range 3–16°, for the O₂, Ar and N₂ plasma treatments compared to the value for the untreated material of 42°. The corresponding value for NH₃ treatment was 25°.

Changes in the apolar contributions to the surface energy were also observed by measuring the advancing and receding angles in 1-bromonaphthalene, Fig. 2b. $\theta_{\text{A}}^{\text{bro}}$ measured on O₂, Ar, N₂ and NH₃ treated surfaces reduced from 46° to values in the range 31–42° and $\theta_{\text{R}}^{\text{bro}}$ changed from 37° to values in the range 25–40°.

Table 3

Statistical significance of differences (*p*-values) between experimental values for the dynamic contact angle data measured in PBS for the following

		Oxygen	Argon	Nitrogen	Ammonia
Virgin ^a	θ_A	4.7×10^{-9}	1.2×10^{-8}	3.1×10^{-6}	2.7×10^{-7}
	θ_R	0.102	0.615	0.093	0.0013
1 month in PBS ^b	θ_A	6.24×10^{-4}	0.87	0.00557	3.37×10^{-5}
	θ_R	0.055	2.25×10^{-7}	0.509	8.31×10^{-6}
1 month in air ^c	θ_A	1.17×10^{-10}	7.67×10^{-8}	7.27×10^{-5}	6.96×10^{-7}
	θ_R	6.48×10^{-5}	0.0149	0.597	8.25×10^{-8}

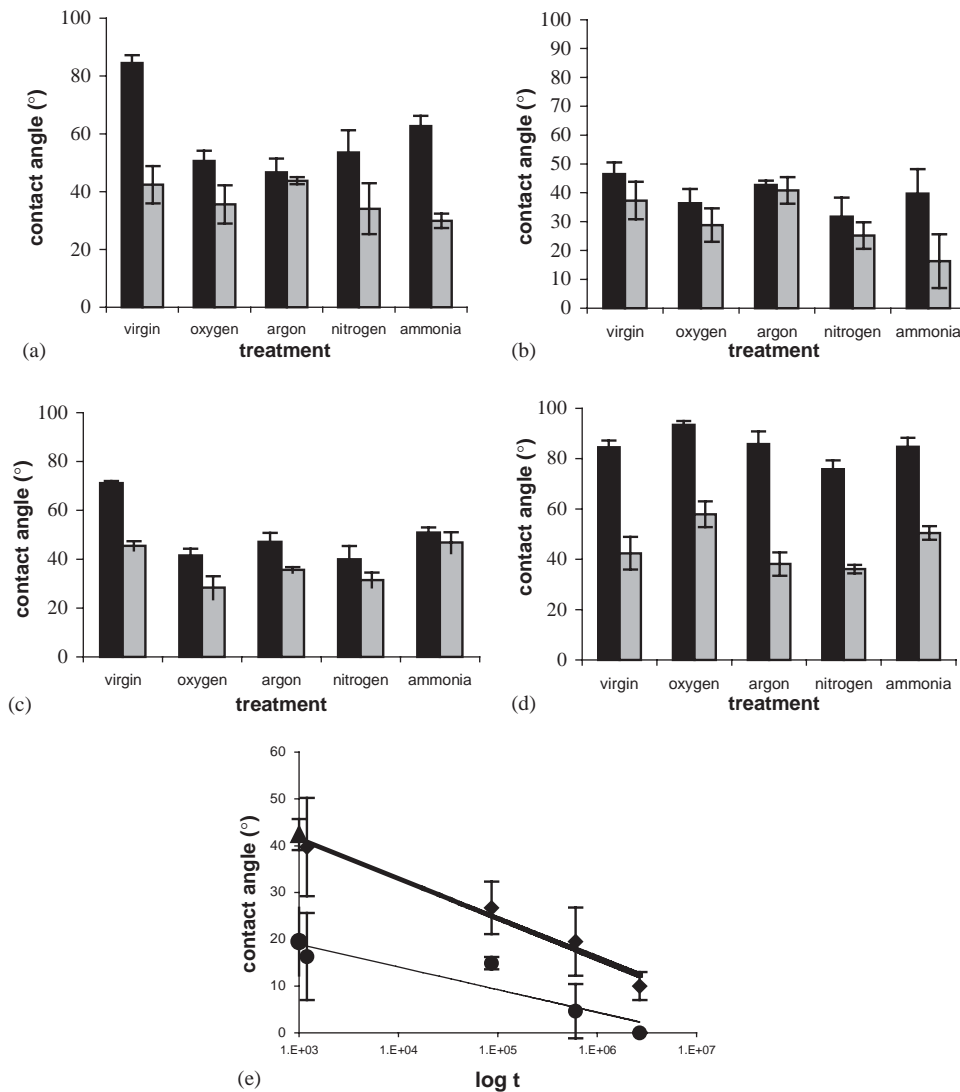
^a Comparison of the advancing and receding angles on the untreated materials with the plasma treated materials.^b Comparison of the advancing and receding angles for each plasma treated material with the same treated material after 1 month storage in PBS.^c Comparison of the advancing and receding angles for each plasma treated material with the same treated material after 1 month storage in air.

Fig. 2. Dynamic contact angle data for virgin and plasma modified PEU surfaces (a) in PBS immediately after treatment; (b) in 1-bromonaphthalene immediately after treatment; (c) in PBS following storage for 1 month in PBS; (d) in PBS following storage for 1 month in air (■ advancing angle, ■ receding angle); (e) the dynamic contact angles for NH₃ treated PEU following storage in PBS up to 1 month measured in 1-bromonaphthalene (— advancing angle, — receding angle). Mean \pm SD, *n* = 6.

Storage in PBS resulted in a small increase in wettability for the untreated, O₂, N₂ and NH₃ plasma treated materials as illustrated by a decrease in θ_A^{pbs} (Fig. 2c). However, the advancing angle measured for Ar processed surfaces stored in the aqueous environment remained essentially unchanged. Storage in PBS also resulted in a decrease in θ_A^{bro} for all treated surfaces to an extent where there was complete wetting, corresponding to an increase in apolar contribution to surface energy. For example, the change in these angles for the ammonia treated surface are presented in Fig. 2e. A similar wetting behaviour value was observed with θ_R^{bro} for all plasma treatments.

Following ageing in air for 1 month the value of θ_A^{pbs} significantly increased (Table 3) for all treated surfaces to within the range 76–93° (Fig. 2d). θ_R^{pbs} also increased after storage in air, most notably in the case of O₂ treatment. Therefore, the θ_A increase for the O₂ plasma treated surface, which exceeds that of untreated PEU, is further indication that the mechanism of modification is restricting the hard segment mobility. Environmental ageing in air had little effect on the apolar characteristics as illustrated by the range of values of θ_A^{bro} (34–47°) and θ_R^{bro} (20–28°) obtained.

3.3. Atomic force microscopy

There is a microphase separation between the hard and soft segments [48] of the untreated PEU (Fig. 3). This incompatibility of the phases allows the unusual physical and mechanical properties associated with PUs [49,50]. The stone like protrusions that are about 0.5 μm in diameter are indicative of the polar domains (crystalline regions), and these appear to adopt a random orientation at the surface. All the plasma treatments modify the surface morphology of the PEU. Fig. 4a illustrates the surface topography following O₂ plasma treatment and indicated the mildest modification within the surface region of all three of the plasmas considered. The resulting surface shows a fine globular texture superimposed onto the original morphology suggesting a mild etching mechanism throughout both the hard and soft segments. The corrugated pattern shown for the Ar plasma in Fig. 4b illustrates pronounced surface modification and/or reorientation of the hard and soft segment domains. For the N₂ and NH₃ plasma treated PEU materials the surface morphology is pitted and results primarily due to etching of the soft segment. The original topography of the parent material is changed significantly for all the plasmas with the Ar plasma having the greatest effect. The observations of the AFM complement the information obtained for the other surface techniques, in that the modification mechanism for each gas is slightly different.

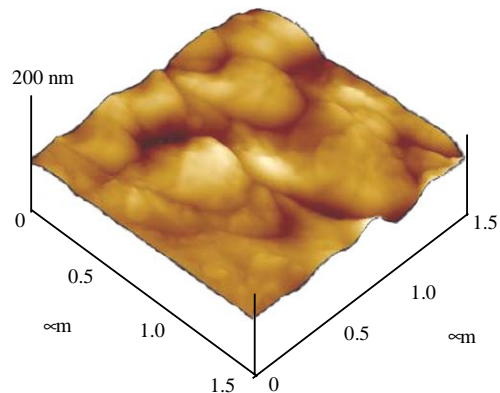


Fig. 3. AFM micrograph of the untreated polyetherurethane surface.

3.4. PTT analysis

The results of the PTT experiment are presented in Fig. 5. The coagulation times of the PPP in contact with all the surfaces lie within the positive and negative controls. The effect of incubating PPP with untreated PEU caused significant contact phase activation, resulting in the coagulation time decreasing from 241 to 170 s ($p = 0.00002$). Plasma treatment in all cases resulted in a decreased level of contact phase activation, the clotting times increasing in comparison with the untreated surface. In particular the N₂ treated surface resulted in an apparent contact phase activation unchanged compared with PPP which has not been in contact with a biomaterial ($p = 0.43$ for difference between negative control and N₂ treatment), the clotting time being 253 s. The N₂ treatment is therefore highly significantly different from the untreated PEU sample ($p = 0.00002$). Whilst the assay was not explicitly calibrated, in other experiments it has been possible to demonstrate a relationship between plasma clotting time and kaolin concentration (Fig. 6), where the response of the activation of fXII with a potent, negatively charged contact activator (kaolin) was observed to be proportional to $\log(\text{kaolin concentration})$ over a range of concentrations from 1 to 1000 $\mu\text{g/ml}$. The rate of unactivated plasma clotting is highly variable between plasma pools, but the slope of $\log(\text{kaolin concentration})$ and clotting time is quite well conserved. One can estimate, therefore, that the difference in contact phase activating power fits approximately to the following equation:

$$[(t_x - t_y)/t_{\text{un}}] \times 5.6,$$

$$\Delta A = 10,$$

where ΔA is the difference in activating power, t_x and t_y are the test clotting times and t_{un} is the negative control clotting time.

The calculation above gives an indication of the activation response relative to untreated PEU due to

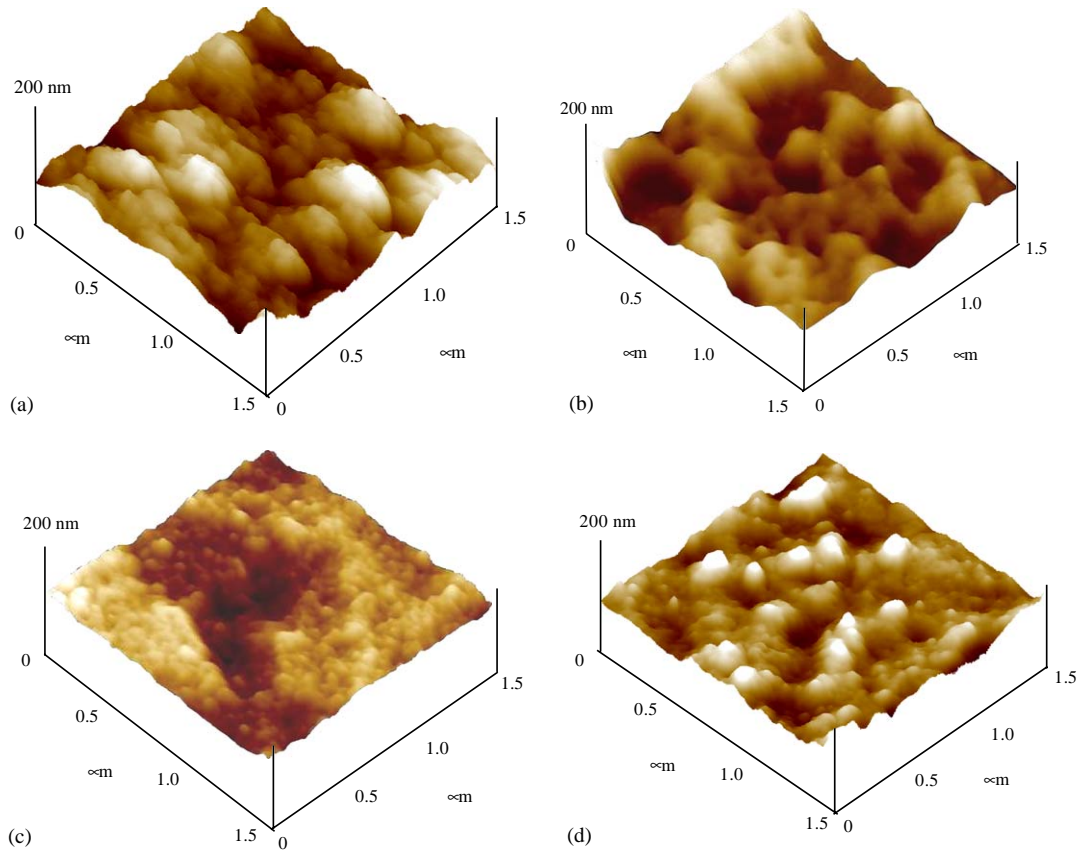


Fig. 4. AFM micrographs of (a) O₂ treated; (b) Ar treated; (c) N₂ treated; (d) NH₃ treated PEU.

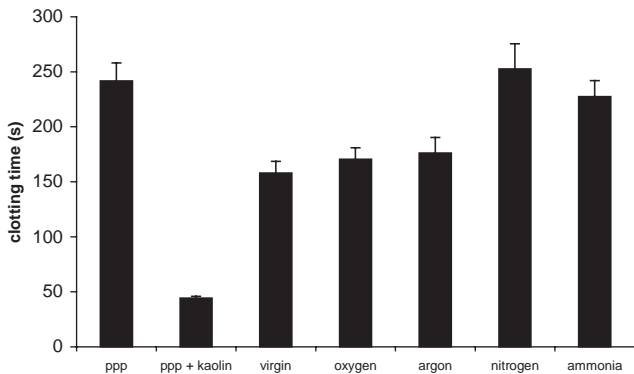


Fig. 5. Partial thromboplastin clotting times of the untreated and plasma treated surfaces in comparison with controls (seconds), Mean \pm SEM, $n = 6$.

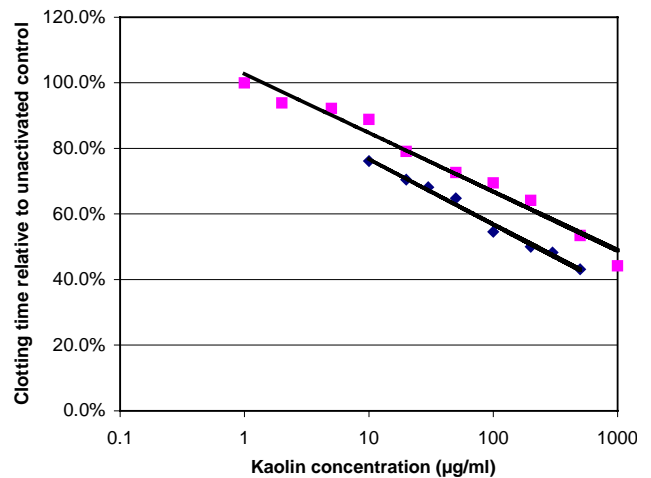


Fig. 6. Relationship between plasma clotting time relative to the negative control and kaolin concentration for two different plasma pools.

incubation of the treated surfaces with PPP, as presented in Table 4.

4. Discussion

The primary objective of this investigation has been to compare the effect of a number of plasma treatments on

a PEU surface used for biomedical applications. Furthermore subsequent ageing in air and PBS at 37°C was monitored to assess the degree of permanency of any changes introduced following plasma treatment. It is further demonstrated that the observed changes in the surface functionality significantly influences the

Table 4
Degree of contact phase activation of each treated surface as a proportion of the untreated PEU following incubation with PPP

Plasma treatment	Proportion of fXII activation relative to untreated PU
O ₂	0.90
Ar	0.81
NH ₃	0.04
N ₂	0.00

blood coagulation response. Consequently, a comparison of the extent of the surface modification and the mechanism involved for each of the charge gases has been hypothesised. Such an understanding is necessary to successfully engineer biomaterial surfaces and the attachment of chemical groups in a controlled and predetermined manner.

4.1. Surface chemistry

The nature of the unmodified materials investigated *in vacuo* by XPS, which provides a model for the behaviour in air, appear to be predominantly governed by relatively non-polar soft segments containing mainly carbon and some oxygen, but only a small amount of nitrogen associated with the hard segment. This observation can be explained by thermodynamic rearrangement at the polymer/air interface and subsequent movement of hard segments into the bulk thus minimising the surface free energy [51]. Therefore in effect, plasma treatment is primarily directed towards the polyether segment of the block copolymer due to the overall predominance of this soft segment in the surface region.

For all the plasma treatments there is a decrease in the relative aliphatic (C–R) character of the material which drops by between 10% and 20% and is most pronounced for nitrogen plasma treatment (Table 2). In all cases the plasma process involves some kind of “modification mechanism” but the combination of additions and subsequent rearrangement of functionalities is different for each of the gases used. From the XPS data obtained it would appear that the mechanism of the Ar and O₂ plasmas is quite similar such that the relative percentage of oxygen introduced into the interfacial layer is increased and carbonyl group formation can also be identified at 288 eV. The slight reduction in the amount of nitrogen to carbon at the surface following both of these plasma treatments suggests that the hard segments are not greatly affected by the energetic species in both cases, but rather a modification of the polyether segment occurs. In the case of N₂ and NH₃ plasmas the relative peak area of the C–O region decreases (Table 2) and can be interpreted as loss of polyether through a plasma

erosion process. At the same time there is an increase of the carbamate group which can be attributed to exposure of deeper lying nitrogen rich functionality. Surface processing using nitrogen gas also results in incorporation of N-moieties as illustrated by the additional peak identified at 287 eV in Fig. 1d, and is assigned to a C=N group. Curve fitting of the C1s region indicated there is formation of highly oxygenated carbon functionality and results in a change in the interfacial properties due to oxidation of the surface [52].

4.2. Wettability

DCA measurements are able to illustrate the surface molecular mobility, surface roughness and surface homogeneity at the polymer/air/water interface. The poor wettability of the untreated surface was supported by a high advancing angle of 84.5° that is fairly typical of that seen in PEUs [52], and reflects the existence of aliphatic carbon on the surface from the soft segment. At the same time the receding angle of 42.2° indicates that there is also a relatively high concentration of polar hard segment at the interface. Therefore, the observed contact angle hysteresis of ~40° indicates the untreated PEU has both hydrophilic and hydrophobic phases residing at the interface, with opposing surface energies. This substantiates the dynamic nature of the material and results in a driving force for rearrangement depending upon the contacting environment of the molecular chains at the surface to minimise the interfacial free energy [53]. A large hysteresis may be observed when there are surface features resulting from etching and mould scars. However, on the basis of the XPS studies the values of θ_A and θ_R support the existence of a microphase separated structure [54] as displayed by the AFM in Fig. 3.

After plasma processing the wettability of treated surfaces increased which correlated well with the measured chemical modification. It has been shown that PU is susceptible to plasma treatment based on the significant decrease in advancing angles observed (Table 3) and has been reported elsewhere [52,55]. If polar groups are being lost through plasma treatment an increase in θ_R would be expected. However, the receding angle does not change substantially and this indicates that plasma treatment is introducing new functionalities such that the net effect of gain and loss is balanced. Therefore, there is an overall decrease in the height of the energy barriers between θ_A and θ_R , i.e. the contact angle hysteresis, of the untreated and plasma treated surfaces (Fig. 2a). In particular, the Ar plasma appears to introduce highly polar groups such as –OH onto the surface so that θ_A decreases to a comparable value with θ_R . In the case of nitrogen treatment there is no as high a concentration of polar groups at the surface since the

contact hysteresis is higher than for Ar and O₂ plasmas, respectively.

4.3. Effect of ageing environments

An important consideration of a modification to surface chemistry is how permanent the changes are. Therefore, the effect of storage times and conditions on the rearrangement and migration of groups following plasma exposure establishes the potential use of these modification in biomedical applications. Storage in PBS does not affect the surface chemistry of the untreated material illustrating that any observed chemical modification in the surface region results from pre-treatment with the plasma gas. The oxygen-containing species introduced following Ar and O₂ treatment are not lost to the aqueous medium over the time period investigated as little change is witnessed in the XPS spectra. In contrast, the N₂ and NH₃ modified PEUs lose most of the nitrogen-containing groups and gain oxygen functionality after 1 month storage in PBS suggesting that these surfaces can reorientate in this medium. Untreated PEU surfaces show a reduction in the contact angle hysteresis following storage in PBS that can be attributed to either water absorption or hard segment enrichment [53,54]. The wettability of plasma treated surfaces stored in the same medium remains essentially unchanged.

The plasma treated PUs that were placed in air for 1 month lose the effects of the surface treatments and become less hydrophilic over time, such that there is an increase in advancing angle to a value similar to the untreated material. This occurs due to the enrichment of the energetically favourable hydrophobic soft segment at the air/polyurethane interface and suggests that some of the polar groups introduced by plasma treatment may be lost under ambient conditions or indeed diffuse into the bulk over time. The driving force for this mechanism comes from the thermodynamic requirement of minimising surface tension. The observed decrease in wettability is also associated with rotational (i.e. torsional) motions of the polymer chains. However, the loss of weakly bound volatile oxygen species cannot be ruled out. The rate of hydrophilicity loss is related to the thermal motion of polymer chains and hydrophilic groups. The effect is particularly pronounced for the O₂ plasma, which shows an increase in θ_A exceeding that of the untreated material. Interfacing the material with a high-energy environment such as PBS causes a change in the reorganisational behaviour since the polar groups introduced are attracted to the surface by its contact with the liquid and is consistent with the findings of the XPS data.

The importance of surface modifications becomes apparent when their interaction with the biological environment is evaluated. Thrombus formation by

foreign surfaces is triggered by contact phase activation of the plasma coagulation pathway. Static contact of PPP with the untreated PEU resulted in a decrease in the clotting time, suggesting a significant material-induced plasma clotting, which would be observed as an increased propensity for thrombus formation when deployed clinically. The O₂ and Ar plasmas produced surfaces, which did not significantly alter the contact activation response compared with the untreated PEU. It can be deduced that the core properties of the PEU with respect to the adsorbed proteins is therefore not changed after treatment with these gases. The ability of a surface to promote contact activation depends on the conformational arrangement of fXII after adsorption, and the assembly of kallikrein and its cofactor high molecular weight kininogen (HMWK). Factor XII is activated to α -fXIIa when the Arg–Val bond between residues 353 and 354 is cleaved and so the rate of plasma clotting will depend on the number of adsorbed fXII molecules that are sterically arranged to allow this cleavage by kallikrein to occur. The generalised observations that negatively charged substances (glass, kaolin, sulfatides, etc. [56,57]) enhance the activation response of fXII point to the manner in which this sterically favourable conformation is achieved. It is therefore interesting that the surfaces that have a promotion of oxygen-bearing groups (the Ar and O₂ plasmas), and therefore a greater propensity to form negative charges at the surface (principally C=O and C–OH) induce the greater contact activation, compared to oxygen depleting and nitrogen enriching surfaces (the N₂ and NH₃ plasmas). The O:N ratios for these surfaces are 7.1, 7.7, 1.33 and 0.75 for O₂, Ar, NH₃ and N₂, respectively. One can speculate, therefore, that when the O:N ratio falls to a threshold value, contact activation is effectively prevented. This needs further investigation to determine the absolute and spatial requirements of the O and N groups for contact activation to occur.

5. Conclusions

The results obtained in this study indicate that plasma modification occurs in a mechanism particular for each gas studied, as exhibited by the different morphologies seen in AFM, but in all cases acts to increase the surface free energy of the PEU. XPS spectra showed that the technique has been successful in the formation of surface species that are not seen in the untreated material, for example the appearance of C=O functionalities for Ar and O₂ treatments, and nitrogen-containing groups for the N₂ plasma. For all the treated surfaces there is an increase in the polar character as displayed by an increase in wettability.

The degree of permanency of each modified surface is dependant on the plasma and storage conditions. For

N₂ and NH₃ plasma treated surfaces there was loss of the introduced groups and subsequent oxidation on storage in an aqueous environment. In contrast, very little change was observed for O₂ and Ar treated surfaces.

The biological response to these surfaces demonstrated that the O₂ and Ar treated surfaces had an insignificant effect on the thrombogenicity of the PEU, but a significant reduction in activation for the NH₃-treated surface and almost complete prevention of the contact activation in the N₂-treated PEU.

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References

- [1] Szycher M. Biostability of polyurethane elastomer: a critical review. In Sharma CP, Szycher M, editors. *Blood compatible materials and devices*. Lancaster, PA: Technomic Publishing Co.; 1990. p. 33.
- [2] Griesser HJ. Degradation of polyurethanes in biomedical applications—a review. *Polym Degrad Stab* 1991;33:329–54.
- [3] Santerre JP, Labow RS. The effect of hard segment size on the hydrolytic stability of polyether-urea-urethanes when exposed to cholesterol esterase. *J Biomed Mater Res* 1997;36:223–32.
- [4] Schubert MA, Wiggins MJ, Schaefer MP, Hiltner A, Anderson JM. Oxidative biodegradation mechanisms of biaxially strained poly(etherurethane) elastomers. *J Biomed Mater Res* 1995;29:337–47.
- [5] Tyler DJ, Ratner BD. Oxidative degradation of Biomer™ fractions prepared by using preparative-scale gel permeation chromatography. *J Biomater Sci Polym Ed* 1994;6:359–73.
- [6] Gunatillake PA, Meijs GF, Chatelier RC, McIntosh DM, Rizzardo E. Synthesis and characterization of hydroxy-terminated poly(alkylene oxides) by condensation polymerization of diols. *Polym Int* 1992;27:275–83.
- [7] Reed AM, Potter J, Szycher M. A solution grade biostable polyurethane elastomer: chronoflex AR. *J Biomater Appl* 1994;8:210–36.
- [8] Leleh MD, Cooper SL. *Polyurethanes in medicine*. Boca Raton, FL, USA: CRC Press; 1986.
- [9] Planck H, Syré I, Dauner M, Egbers G, editors. *Polyurethane in biomedical engineering. II. Progress in biomedical engineering*, vol. 3. Amsterdam: Elsevier Science; 1987.
- [10] Silver DF, Hempling RE, Recio FO, Piver MS, Eltabbakh GH. Complications related to indwelling caval catheters on a gynecologic oncology service. *Gynecol Oncol* 1998;70:329–33.
- [11] Lewis GBH, Hecker JF. Infusion thrombophlebitis. *Br J Anaesth* 1985;57:220–33.
- [12] Collin J, Collin C, Constable FL, Johnston IDA. Infusion thrombophlebitis and infection with various cannulas. *Lancet* 1975;2:150–3.
- [13] Nightingale CE, Norman A, Cunningham D, Young J, Webb A, Filshie J. A prospective analysis of 949 long-term central venous access catheters for ambulatory chemotherapy in patients with gastrointestinal malignancy. *Eur J Cancer* 1997;33:398–403.
- [14] Mughal MM. Complications of intravenous feeding catheters. *Br J Surg* 1989;76:15–21.
- [15] Dollery CM, Sullivan ID, Bauralnd O, Bull C, Milla PJ. Thrombosis and embolism in longterm central venous access for parenteral nutrition. *Lancet* 1994;344:1043–5.
- [16] Mahutte CK, Sassoon CSH, Muro JR, Hansmann DR, Maxwell TR, Miller WW, Yafuso M. Progress in the development of a fluorescent intravascular blood-gas system in man. *J Clin Monitor* 1990;6:147–57.
- [17] Pollard AJ, Sreeram N, Wright JG, Beath SV, Booth IW, Kelly DA. ECG and echocardiographic diagnosis of pulmonary thromboembolism associated with central venous lines. *Arch Dis Child* 1995;73:147–50.
- [18] Glaser DW, Medeiros D, Rollins N, Buchanan GR. Catheter-related thrombosis in children with cancer. *J Pediatr* 2001;138:255–9.
- [19] Silverberg M, Dunn JT, Garen L, Kaplan AP. Autoactivation of human Hageman factor. demonstration utilizing a synthetic substrate. *J Biol Chem* 1980;255:7281–6.
- [20] Didisheim P, Mibashan RS. Activation of Hageman factor (factor XII) by long chain saturated fatty acids. *Thromb Diath Haemorrh* 1963;9:346–53.
- [21] Kellermeyer RW, Breckenridge RT. The inflammatory process in acute gouty arthritis. I. Activation of Hageman factor by sodium urate crystals. *J Lab Clin Med* 1965;65:307–15.
- [22] Borow M, Crowley JG. Evaluation of central venous catheter thrombogenicity. *Acta Anaesth Scand* 1985;Suppl. 81:59–64.
- [23] Larm O, Larsson R, et al. A new non-thrombogenic surface prepared by selective covalent binding of heparin via a modified reducing terminal residue. *Biomater Med Devices Artif Organs* 1983;11:161–73.
- [24] Park KD, Okano T, Nojiri C, Kim SW. Heparin immobilization onto segmented polyurethane-urea surfaces—effect of hydrophilic spacers. *J Biomed Mater Res* 1988;22:977–92.
- [25] Hayward JA, Durrani AA, Lu Y, Clayton CR, Chapman D. Biomembranes as models for polymer surfaces. IV. ESCA analyses of a phosphorylcholine surface covalently bound to hydroxylated substrates. *Biomaterials* 1986;7:252–8.
- [26] Guidoin RG, Awad J, Brassard A, et al. Blood compatibility of silicone rubber chemically coated with cross-linked albumin. *Biomater Med Devices Artif Organs* 1976;4:205–24.
- [27] Campbell DJ, Dixon B, Kladis A, Kemme M, Santamaria JD. Activation of the kallikrein-kinin system by cardiopulmonary bypass in humans. *Am J Physiol* 2001;281:R1059–70.
- [28] Campbell DJ. The kallikrein-kinin system in humans. *Clin Exp Pharmacol Physiol* 2001;28:1060–5.
- [29] Schmaier AH. The plasma-kallikrein-kinin system counterbalances the rennin-angiotensin system. *J Clin Invest* 2002;109:1007–9.
- [30] Rodén L. *Heparin*. London: Edward Arnold, 1989. p. 240.
- [31] Hirsh J, Heparin N. *Engl J Med* 1991;324:1565–74.
- [32] Halm MA. Acute gastrointestinal complications after cardiac surgery. *Am J Crit Care* 1996;5:109–18.
- [33] Dutton RC, Edmunds LH, Hutchinson JC, Roe BB. Platelet aggregate emboli produced in patients during cardiopulmonary bypass with membrane and bubble oxygenators and blood filters. *J Thorac Cardiovasc Surg* 1974;67:258–65.
- [34] Garbassi F. Polymer surfaces from physics to technology. In: Morra M, Occhiello E, editors. *Spectroscopic methods*. Chichester: Wiley; 1994. p. 98.
- [35] Hansen R, Pascale J. Effect of atomic oxygen on polymers. *J Polym Sci A* 1965;3:2205–22.

- [36] Clark DT. ESCA applied to polymers XVIII RF glow discharge modification of polymers in helium, neon argon and krypton. *J Polym Sci Polym Chem Ed* 1978;16:911–36.
- [37] O' Kell S, Henshaw T, Farrow G, Aindow M, Jones C. Effects of low-power plasma treatment on polyethylene surfaces. *Surf Interface Anal* 1995;23:319–27.
- [38] Pringle SD, Joss VS, Jones C. Ammonia plasma treatment of PTFE under known plasma conditions. *Surf Interface Anal* 1996;24:821–9.
- [39] Hollahan J, Carlson G. Hydroxylation of polymethylsiloxane surface by oxidizing plasmas. *J Appl Polym Sci* 1970;14:2499–508.
- [40] Santerre JP, Labow RS, Duguay DG, Erfle D, Adams GA. Biodegradation evaluation of polyether and polyester-urethane with oxidative and hydrolytic enzymes. *J Biomed Mater Res* 1994;38:1187–99.
- [41] Lander LM, Siewierski LM, Brittain WJ, Vogler EA. A systematic comparison of contact angle methods. *Langmuir* 1993;9:2237–9.
- [42] Galuska AA, Poulter RR, McElrath KO. Force modulation AFM of elastomer blends: morphology, fillers and cross-linking. *Macromolecules* 1991;28:1108–14.
- [43] Magonov SN, Reneker DH. Characterisation of polymer surfaces with atomic force microscopy. *Annu Rev Mater Sci* 1997;27:175–222.
- [44] Rhodes NP, Williams DF. Plasma recalcification as a measure of contact phase activation and heparin efficacy after contact with biomaterials. *Biomaterials* 1994;15:35–8.
- [45] Sabbatini L, Zambonin PG. XPS and SIMS surface chemical analysis of some important classes of polymeric biomaterials. *J Electron Spectrosc* 1996;81:285–301.
- [46] Tieszer C, Reid G, Denstedt J. XPS and SEM detection of surface changes on 64 ureteral stents after human usage. *J Biomed Mater Res* 1998;43:321–30.
- [47] Vargo TG, Hook DJ, Gardella JA, Eberhardt MA, Meyer AE, Baier RE. A surface spectroscopic and wettability study of a segmented block copolymer poly(etherurethane). *Appl Spectrosc* 1991;45:448–56.
- [48] Xu MX, Liu WG, Wang CL, Gao ZX, Yao KD. Surface crystalline characteristics of polyurethane investigated by atomic force microscopy. *J Appl Polym Sci* 1996;61:2225–8.
- [49] Christenson CP, Harthcock MA, Meadows MD, Spell HL, Howard WL, Creswick MW, Guerra ME, Turner RB. Model MDI butanediol polyurethanes—molecular structure, morphology, physical and mechanical properties. *J Polym Sci B* 1986;24:1401–39.
- [50] Wang CS, Kenney DJ. Effect of hard segments on morphology and properties of thermoplastic polyurethanes. *J Elastom Plast* 1995;27:182–99.
- [51] Kober M, Wesslen B. Surface-properties of a segmented polyurethane containing amphiphilic polymers as additives. *J Appl Polym Sci* 1994;54:793–803.
- [52] Li DJ, Zhao J. Surface biomedical effects of plasma on polyetherurethane. *J Adhes Sci Technol* 1995;9:1249–61.
- [53] Yasuda H, Charlson EJ, Charlson EM. Dynamics of surface property change in response to changes in environmental conditions. *Langmuir* 1991;7:2394–400.
- [54] Tingey KG, Andrade JD. Probing surface microheterogeneity of poly(ether)urethanes in an aqueous environment. *Langmuir* 1991;7:2471–8.
- [55] Giroux TA, Cooper SL. Surface characterization of plasma-derivatised polyurethanes. *J Appl Polym Sci* 1991;43:145–55.
- [56] Tankersley DL, Finlayson JS. Kinetics of activation and autoactivation of human Factor XII. *Biochemistry* 1984;23:273–9.
- [57] Tans G, Griffin JH. Properties of sulfatides in factor-XII-dependent contact activation. *Blood* 1982;59:69–75.