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Biomaterials 28 (2007) 4561–4570
Biomaterials
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The effect of gas plasma modification on platelet and contact phase activation processes

Nicholas P. Rhodes[a,b], Darren J. Wilson[a,1], Rachel L. Williams[a,*]

[a]Clinical Engineering, School of Clinical Sciences, University of Liverpool, Daulby Street, Liverpool L69 3GA, UK
[b]UK Centre for Tissue Engineering, University of Liverpool, Daulby Street, Liverpool L69 3GA, UK

Received 3 April 2007; accepted 4 July 2007
Available online 31 July 2007

Abstract

Medical-grade polytetrafluoroethylene (PTFE), polydimethylsiloxane (PDMS), polyetherurethane (PEU) and ultrahigh molecular weight polyethylene (UHMWPE) were plasma treated with O_2, Ar, N_2 and NH_3. Their surface properties were characterised using X-ray photoelectron spectroscopy (XPS), static secondary ion mass spectroscopy (SSIMS), atomic force microscopy (AFM) and dynamic contact angle (DCA) analysis. Platelet adhesion, aggregation, activation and release of microparticles were determined after contact with whole blood in a cone and plate viscometer. Activation of the coagulation system was quantified in a static environment using a partial thromboplastin time (PTT) assay. The chemical compositions of the untreated surfaces were found to be very similar to those of the bulk material except for PEU, whose surface was comprised almost entirely of soft ether segments. For all materials, the different plasma treatments resulted in moderate etching with the incorporation of functional groups and removal of side groups: defluorination, dehydrogenation, cleavage of methyl side groups and soft segments for PTFE, UHMWPE, PDMS and PEU, respectively. Consequently, plasma treatment resulted in increased wettability in all cases. Blood contact with the virgin materials resulted in activation of platelets and the clotting cascade. Plasma treatment resulted in a significant reduction in platelet adhesion for all materials and all treatments. In the case of PTFE and PEU, the activation status of these cells was also reduced. Plasma treatment of all materials reduced fluid-phase CD62P expression. Platelet aggregate size correlated well with degree of aggregate formation, but many treatments increased the degree of aggregation, as was the case for microparticle shedding. There was no correlation between CD62P expression, aggregate formation and platelet microparticle (PMP) shedding. It is concluded that despite incorporation of the same chemical groups, the pattern of response to blood *in vitro* is not the same across different polymers.

Keywords: Polymers; Haemocompatibility; Platelet; Microparticle; Coagulation

1. Introduction

Biomaterial-related thromboembolic complications remain a significant risk in the deployment of blood-contacting biomaterials [1,2]. Despite many studies of interfacial phenomena of biomaterials in blood, no generalised theory relating surface physico-chemical characteristics to performance in blood has to date been established. Although some early efforts attempted to correlate blood compatibility parameters with material performance [3–5], exceptions which do not follow any such correlation are commonly observed, which prevent predictive biomaterial engineering. Furthermore, attempts to reduce the impact of the material on the response to blood using conventional chemical means are often fruitless [6–8].

Most blood-contacting devices are constructed from common polymeric materials taken directly from applications unrelated to surgery [9–11]. Due to mass production, these 'standard' materials can be produced economically and for many medical applications they can be used quite satisfactorily. An ever-increasing desire to improve the performance of blood-contacting devices in certain demanding applications has resulted in the creation of

*Corresponding author. Tel.: +44 151 706 4211; fax: +44 151 706 4915.
E-mail address: npr@liv.ac.uk (R.L. Williams).
[1]Current address: Smith and Nephew Group Research Centre, York Science Park, York Y10 5DF, UK.

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doi:10.1016/j.biomaterials.2007.07.008

technically advanced, but expensive to manufacture, solutions often involving drug-delivery systems or pharmaco-combinatorial devices. However, it may be possible to create simple surface chemistries which are effective in resolving many of the observed incompatible reactions.

One common approach for producing a material with a range of different surface properties is gas plasma treatment [12]. By careful attention to the processing conditions, there is some control over chemical specificity enabling the surface chemistry of a polymer to be tailored for a particular application. Such modifications can be performed reliably and economically as part of a mass-production process. Plasma treatment in O_2 and Ar gases frequently result in the incorporation of oxygen-containing groups [13,14], whereas N_2 and NH_3 treatments incorporate both nitrogen and oxygen-containing groups depending on whether the treated surface is subsequently exposed to air [15,16]. In addition, the surface roughness of polymeric surfaces can be modified by ablation of chain segments [17], the extent of which is dependent upon the plasma system used. Owing to the unstable nature of treated surfaces in some plasma systems, post-treatment reactions that stabilise surface chemical modifications are important [12].

Initial studies of nitrogen and ammonia plasma-treated polyetherurethane (PEU) and polydimethylsiloxane (PDMS) have indicated that the activation of the coagulation cascade is reduced compared to untreated surfaces [18,19]. It may be hypothesised, therefore, that it could be possible to induce blood-contact phenomena by surface treatments using low-powered plasmas that benefited a wider range of biological pathways. This hypothesis has been tested in this study.

The first objective was to describe the surface changes for ultrahigh molecular weight polyethylene (UHMWPE), polytetrafluoroethylene (PTFE), PDMS and PEU due to O_2, Ar, N_2 and NH_3 gas plasma treatments followed by a post-treatment reaction in distilled water, based on the hypothesis that certain plasma treatments may induce similar physico-chemical and biological responses on chemically disparate materials. The characterisation of the surfaces comprised X-ray photoelectron spectroscopy (XPS), static secondary ion mass spectroscopy (SSIMS), dynamic contact angle (DCA) measurement and atomic force microscopy (AFM). The second objective was to investigate the haemocompatibility of the untreated and plasma-treated surfaces under *in vitro* conditions using a comprehensive group of platelet indices and activation of the coagulation cascade.

2. Materials and methods

2.1. Materials

UHMWPE and PTFE homopolymers that were essentially free of additives were purchased in sheet form (1 mm thick) from Goodfellow Ltd. (Huntingdon, UK). Two-mm-thick sheets of medical-grade PDMS elastomer $[(CH_3)_2SiO)_n]$ were obtained from A Little John (type MED-4050, Cowbridge, UK), and contained fillers and vulcanising agents, but no other additives. An additive free, medical grade, thermoplastic, aromatic PEU was purchased from Eurothane (Worcester, UK) in 2-mm-thick sheets, comprising alternating diphenyl-methane 4,4-diisocyanate (MDI) hard and polytetramethylene oxide (PTMO) soft segments with butanediol as the chain extender. Each piece of material was cut into rectangular pieces (10×20 mm) for physico-chemical studies and round coupons 25 mm in diameter for blood-contacting studies. Each specimen was cleaned using diethyl ether to remove surface contaminants and low molecular weight species before plasma treatment. Gases were purchased from BOC (Guildford, UK) as ultrapurity grade: O_2: 99.5%; Ar: 99.998%; N_2: 99.998% and NH_3: 99.99% and were used as supplied.

2.2. Plasma treatment

The rf equipment and treatment procedure in this study has been described in detail previously [15,16]. Briefly, a low powered plasma was produced in a half-wave helical resonator at an excitation frequency of 13.6 MHz. Surface treatment was effected within an atmosphere of one of four gases (O_2, Ar, N_2 or NH_3) at a pressure of 8×10^{-2} mbar and flow rate at 85 sccm for 1 min. Immediately following treatment the samples were placed in distilled water and stored for at least 24 h prior to analysis.

2.3. Surface characterisation

2.3.1. X-ray photoelectron spectroscopy (XPS)

XPS was performed using a VG Scientific ESCALAB MXII spectrometer (East Grinstead, UK) operated at 12 kV and 240 W with an unmonochromated AlKα radiation for sample excitation and take-off angle of 45°. High-resolution spectra were performed in the sequence: C-1s, Si-2p, O-1s, N-1s and a repetition of C-1s to ensure minimal surface radiation damage had occurred. Charging of the surface was not controlled since no flood gun/screen technique was available. Binding energy references utilised the C-1s component of C–Si–O–Si (284.7 eV) in PDMS, the C-1s hydrocarbon peak (285 eV) for PEU, the $–CF_2–$ C-1s signal (291.8 eV) for PTFE and $–CH_2–$ C-1s (285.2 eV) for UHMWPE. Valence band spectra in the range 0–40 eV were acquired using a monochromated XPS spectrometer (Shimadzu Kratos AXIS l, Rydalmere, NSW, Australia) at the same settings. Atomic composition data was carried out using empirical sensitivity factors and all experimental peaks were integrated after non-linear background subtraction using the software provided.

2.3.2. Static secondary ion mass spectroscopy (SSIMS)

SSIMS experiments were carried out using a Millbrook Chemical Microscope (Blackburn, UK). The defocused primary beam comprised 7 keV gallium ions with d.c. current of 2 nA, producing an incident flux of 1.25×10^{10} ions s^{-1} cm^{-2}. The total dose was restricted to 1.3×10^{12} gallium ions cm^{-2} during collection of each spectrum, less than the threshold value of 10^{13} ions cm^{-2} previously reported for extensive surface damage to occur [20]. Both positive and negative spectra were acquired, the m/z range being 2–250 Da in each case, dwell time of 0.01 s per mass channel and step size 0.2 Da. The ratios of the corresponding pairs of spectral peak areas defined the approximate relative abundance of any particular chemical group on untreated compared with plasma-treated surface.

2.3.3. Contact angle measurements

DCA measurements were performed using the Wilhelmy plate technique with a computer-controlled Cahn Instruments DCA322 (Madison, WI, USA) DCA analyser. Mean values of advancing and receding angles (θ_A and θ_R, respectively) were determined from data on at least five independent specimens. Contact angle measurements were carried out in 10 mM phosphate-buffered saline (PBS) pH 7.4 (Aldrich, Poole, UK).

2.3.4. Atomic force microscopy (AFM)

AFM was used in 'tapping mode' to determine changes in micro-roughness induced by plasma treatment, using a Digital Instruments Nanoscope III (Santa Barbara, CA, USA) having a sharp silicon nanosensor attached to a flexible silicon nitride microcantilever. Scans were acquired at room temperature in air at a rate of 1–2 Hz and using two scan angles, 0° and 90° to ensure surface measurements were reliable. The root mean-squared (RMS) roughness and relative surface area, compared to a flat surface, was determined on at least five independent areas of each surface, using the image processing features of the microscope software. Roughness and increase in surface area was measured by evaluating the relative elevations, or z values, and their quantity within a given area of test sample.

2.4. Collection of blood

Blood was withdrawn from healthy male, adult volunteers, who had been free of medication for at least 14 days and had fasted for at least 4 h. Collection was achieved via venous puncture of a median cubical vein using a sterile 19-G needle without the use of a tourniquet. The blood was anticoagulated with *tri*-sodium citrate (3.8% w/v in H_2O) at a volume ratio of 1 part citrate to 9 parts blood.

2.5. Preparation of platelet-poor plasma (PPP)

Contact with other 'foreign' materials was minimised during PPP preparation. Whole blood was centrifuged at 800g for 10 min. The plasma supernatant was carefully extracted and decanted into 1.5 ml Eppendorf tubes which was then spun at 13000g for 30 s in a microcentrifuge. Plasma was carefully removed from each tube, leaving behind any platelet sediment. Plasma which appeared lipaemic was always discarded. Plasma from at least three donors was pooled. From this combined pool, 250 μl of PPP were then placed into 500 μl eppendorf tubes and snap frozen in liquid nitrogen. Tubes were stored at −80 °C until used. For material contact, tubes were warmed at 37 °C in a water bath for precisely 60 s.

2.6. Platelet studies

The analysis of the test materials for the effect on the stimulation of platelets in whole blood has been described in detail previously [21–24]. Briefly, fresh, human whole blood was exposed to each material at a shear rate of 500 s^{-1} for 100 s in a cone and plate rheometer. Fluid-phase platelets were stained with antibodies for CD41 and CD62P so as to determine platelet α-granule secretion, aggregation and microparticle (PMP) shedding. The activation state of adherent platelets was determined using the classification described by Ko and Cooper [25] to determine five stages of platelet shape change from discoid to fully spread. Surfaces were washed with 0.1 M PBS, pH 7.4, to remove non-adherent/loosely bound platelets and red blood cells and then fixed in 2.5% (v/v) gluteraldehyde (Emsope, Ashford, UK) for 30 min at room temperature and dehydrated in 70% (v/v), 90% (v/v) and 100% (v/v) ethanol (HPLC grade, Merck, UK) for 10 min in each concentration. Samples were dried under vacuum, sputter-coated with gold and then observed using SEM. Images of at least five different regions were analysed by image analysis.

2.7. Contact phase activation

The activation of the coagulation system was determined using a partial thromboplastin time (PTT) assay, similar to that described previously [26]. Two hundred microlitres of prewarmed PPP were placed onto the material to be tested and incubated for 10 min at 37 °C in a humidity >95% to prevent plasma evaporation. One hundred microlitres of PPP were then removed and pipetted into an automated coagulometer (Instrumentation Laboratory ACL300 Research, Milan, Italy), where plasma clotting was initiated by the addition of an equal volume of 25 mM $CaCl_2$ and platelet substitute (Diagen reagent, Diagnostic Reagents, Thame, UK). Light absorbance of each sample was determined every 100 for 400 s against a synthetic optical reference. The start of clotting was defined as the time taken for the absorbance to equal 3% of the difference between the initial solution and the optical reference and was termed the PTT. The activating power of a material was estimated by extrapolation of a kaolin (Sigma, Poole, UK) standard curve (logarithmic scale) in the range 2–1000 mg l^{-1} in barbitone buffer (24 mM 5,5-diethylbarbituric acid, 73 mM tris–(hydroxymethyl) aminomethane, 0.79 M calcium lactate, 2.5 mM sodium azide, pH 7.4). In this case, 65 μl PPP was incubated with 35 μl of kaolin for 10 min prior to determination of activation.

2.8. Statistical analysis

The statistical significance of difference between mean values for the dynamic blood contact experiments was determined using ANOVA corrected using a Bonferroni procedure to examine relationships among the data. For all statistical tests carried out, significance was defined as $p<0.05$ (at the 95% confidence interval level); the sample size, n, was $\geqslant 8$ in each case.

3. Results

3.1. Physico-chemical characterisation

Detailed characterisation of the plasma treatments of PTFE, PDMS and PEU have been described already [18,19,27,28] and will not be repeated here. All untreated materials were hydrophobic as determined by their advancing angles. All treatments on all materials caused an increase in hydrophilicity (Fig. 1). The plasma treatments caused a similar trend in the different materials. For all surfaces, the contact angle hysteresis (θ_A–θ_R) was apparent and in the range 20–40° in all cases. Furthermore, these changes in wettability were linked to the incorporation of polar functional groups in the surfaces.

The ratios of elements at the surface of the untreated UHMWPE as determined by XPS were similar to theoretical values and were influenced by plasma treatment (Fig. 2), characterised by dehydrogenation at a proportion of approximately 15–25% with the incorporation of oxygen in place of the displaced hydrogen for all plasma gases and, additionally, inclusion of nitrogen moieties following N_2 and NH_3 plasma treatments. The introduction of oxygen was confirmed by emergence of O-2s electrons in the valence band spectra and detection of $(OH)^-$ and $(O)^-$ in treated UHMWPE by SSIMS. The level of surface oxygen incorporation into NH_3-treated PE was greater than that of the O_2 treatment. The effect of plasma treatment was to remove original side groups and substitution by more polar entities such as hydroxyl, carbonyl and carboxylic groups through reaction with O, OH and O_2 plasma species in the case of oxygen treatment. In the case of NH_3 treatment, nitrogen-containing moieties (amine, imines and nitriles) were introduced instead. However, on exposure to air during sample transfer, and during aqueous incubation, all samples experienced physisorption and chemisorption of oxygen and water molecules leading to increased functionality. All plasma treatments resulted in light etching (Fig. 3) as shown by an increase in

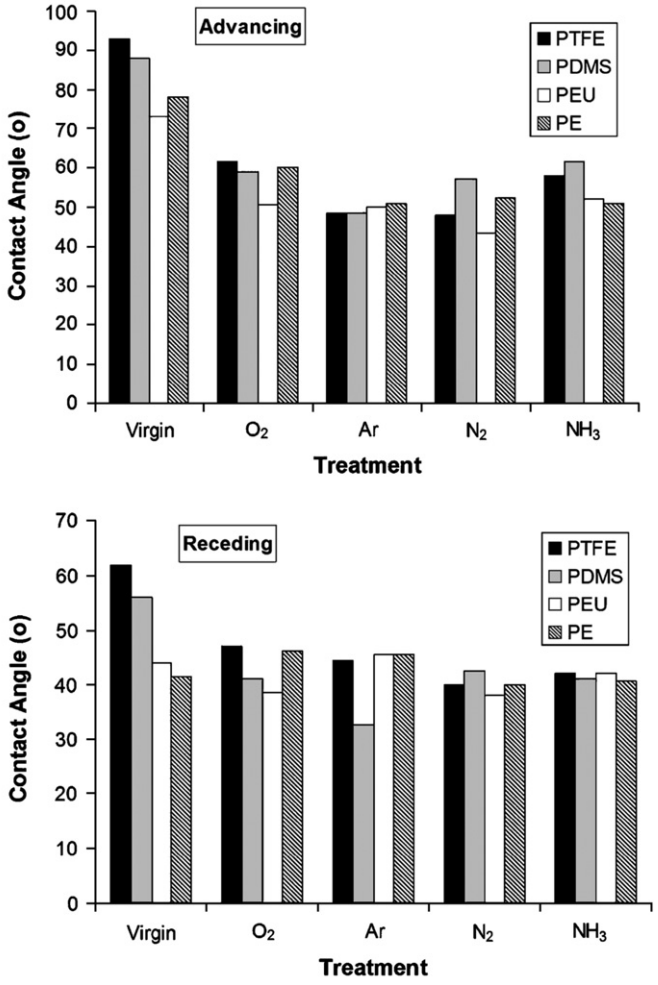

Fig. 1. Advancing and receding contact angles for untreated and plasma-treated polymers after storage in PBS for 24 h.

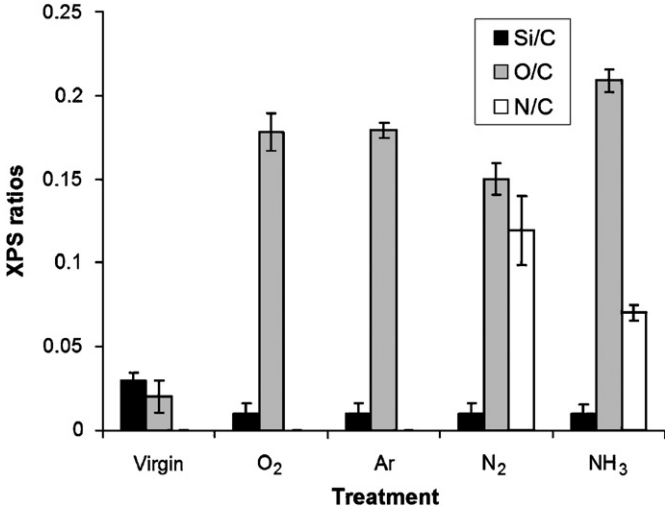

Fig. 2. Elemental ratios determined by XPS for untreated and plasma-treated UHMWPE after storage in PBS for 24 h.

surface area in the range 4–7%. This compared to mean enhancements of 8%, 3% and 9.5% across the treatments for PTFE, PEU and PDMS, respectively. In general, the macroscopic surface topography of UHMEPE was maintained after treatment.

There was evidence of some surface contamination at the surface of untreated materials caused during manufacture when analysed by XPS and SSIMS in the form of Si which could not be removed by the cleaning protocol, and an indication that this was substantially reduced as a result of plasma treatment. This was also observed with polyurethane.

3.2. Adhered platelets

Typical morphology of adhered platelets on the untreated polymers after dynamic contact is shown in Fig. 4. The number of platelets attaching to unmodified polymers was greatest on PEU and PDMS and least on UHMWPE and PTFE (Fig. 5). The high magnification images demonstrate that the adhered platelets were most activated in PTFE and PEU, then UHMWPE and least in PDMS (Table 1). Following plasma treatment, platelet adhesion was significantly reduced on all polymers with all treatments (Figs. 5 and 6). Furthermore, it can be seen that on the more activating polymers (PTFE and PEU), Ar and N_2 treatments resulted in reduced activation of adhered platelets (Fig. 6 and Table 1), differences which were less apparent or absent when modified with NH_3 and O_2 (Table 1). For PDMS and UHMWPE, where activation of adhered platelets was not severe on the untreated polymers, plasma treatments had no obvious effect on their activation status.

3.3. Fluid-phase platelet activation, aggregation and PMP shedding

The degree of CD62P (Fig. 7) expression on fluid-phase platelets was least after dynamic contact with PEU and greatest after PTFE and PDMS contact. The effect of all plasma treatments on all materials was to reduce the CD62P expression, except one (Ar treatment in UHMWPE). There was no apparent trend to the effect of treatment on this expression. All modifications of PTFE, except O_2, and of PDMS, except NH_3 were highly significant ($p \leqslant 0.01$).

The degree of platelet aggregation (i.e. numbers of platelets within aggregates as a proportion of the total) generally correlated with the mean aggregate size (Fig. 8), except for PDMS. This implies that in strict numerical terms the number of platelet aggregates was limited, and single platelets were recruited to ever-expanding aggregates. Aggregation was not related to fluid-phase CD62P expression, and was of similar magnitude after contact with all untreated samples. Many treatments had the effect of halving the degree of aggregation, and the mean aggregate size, but the plasma gas had the same chance of reducing aggregation as it had of having no effect. The reduced aggregation due to treatments of Ar, N_2 and NH_3 on

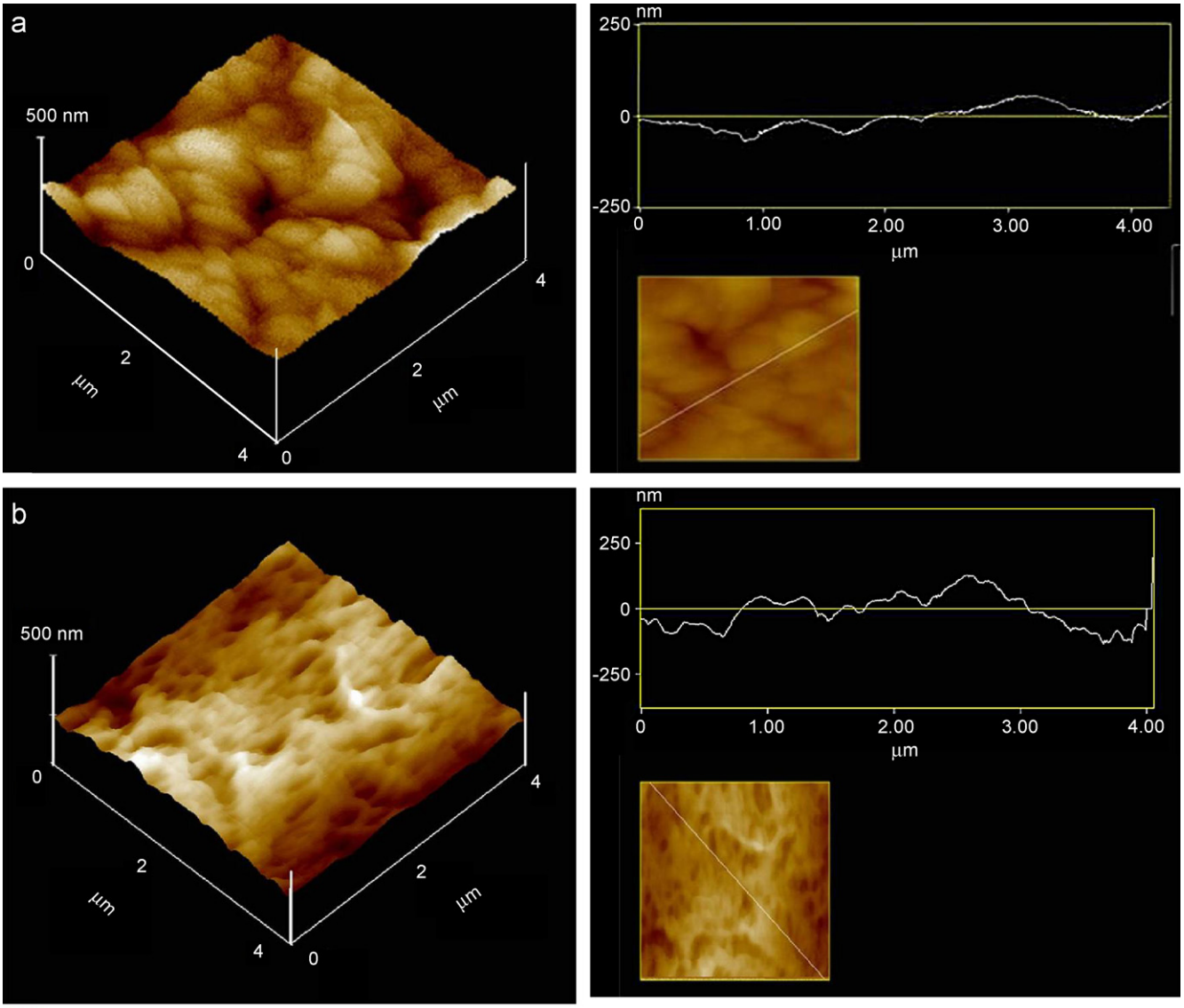

Fig. 3. AFM micrographs of (a) untreated and (b) Ar-treated UHMWPE. Scan angle, $\phi = 0^{\circ}$.

PTFE, N_2 and NH_3 on PDMS and O_2 and Ar on PEU were highly significant ($p < 0.001$ in the case of Ar).

Microparticle shedding was as likely to be unchanged or increased by plasma treatment as it was to be reduced (Fig. 9), but was observed at its least after contact with PTFE. Three treatments significantly reduced shedding on PDMS (O_2, Ar and N_2), two on UHMWPE (Ar and NH_3), whereas NH_3 treatment of PEU and N_2 treatment of UHMWPE increased shedding. It was not correlated to CD62P expression or aggregation.

3.4. Contact phase activation

The effect of material contact on the activation of the intrinsic coagulation cascade is shown in Fig. 10, extrapolated from the activation of kaolin standards and effectively in units of mg ml^{-1} kaolin equivalent activating power. All virgin polymers caused activation which was broadly similar, but in the following ranking: PTFE, PDMS, UHMWPE and PEU (lowest). All treatments of UHMWPE and PEU reduced the activating potential of the virgin material, as did O_2 and N_2 treatments on PTFE and N_2 and NH_3 treatments on PDMS. Reduction in activation due to N_2 and NH_3 treatments on both PEU and PDMS were highly significant. NH_3 modification of UHMWPE was also significant. The most dramatic was N_2 plasma-treatment of PEU which resulted in activation of the coagulation cascade 0.005 times that of virgin PEU ($p < 0.0001$).

4. Discussion

Despite the diverse nature of many of the commonly used blood-contacting biomaterials, it is often noted that the response (e.g. platelet reactions, clotting, etc.) is surprisingly similar. This has been termed the *blah* response

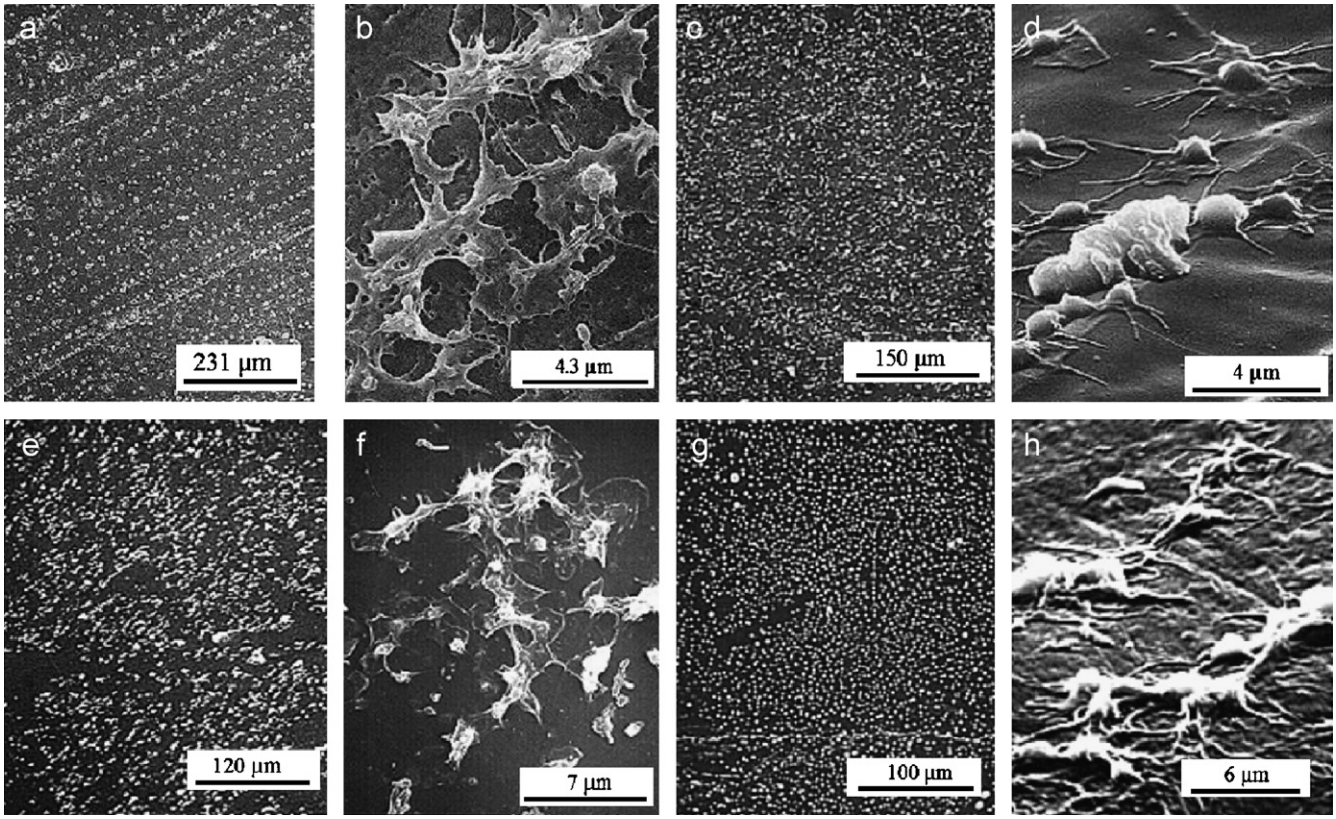

Fig. 4. SEM micrographs of platelets adhered onto untreated polymers after exposure to 500 s^{-1} for 100s: (a, b) PTFE; (c, d) PDMS; (e, f) PEU; and (g, h) UHMWPE.

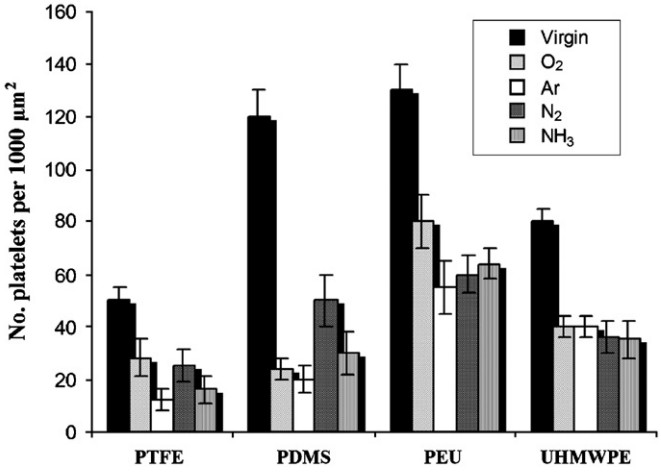

Fig. 5. Numbers of platelets adhered onto untreated and plasma-treated materials.

by Ratner [9], and suggests a high degree of random interaction (protein species, orientation, conformation) between polymeric surfaces and the plasma proteins with which host cells react. Indeed, Ratner suggests that epitaxial ordering of the adsorbed protein layer would allow engineering of the response, such that it may be expected that reactions at the protein or cellular level may be controlled. This study was designed to take four well-characterised polymers, modify their surfaces with simple

Table 1
Qualitative assessment scores of activation status of adhered platelets, from 1 (unactivated) to 5 (highly activated), from Ko and Cooper [25]

	Untreated	Ar	N_2	NH_3	O_2
UHMWPE	2–3	2–3	2–3	2–3	2–3
PTFE	3–5	2	2	3	4
PEU	3–4	2	1–5	3	2–3
PDMS	1–1.5	1–2	1–2	1–2	1–2

1: round; 2: dendritic; 3: spread-denditric; 4: spreading; 5: fully spread.

reactive plasmas which result in the addition of atoms to the surface in a well-characterised manner having similarity for different polymers and then evaluate the biological response to these surfaces in terms of haemocompatibility. Thus, it might be expected that observations of common trends regarding the biological response would be possible. Initial studies [18,19,28] demonstrated that all these plasmas had the effect of side-chain substitution on all the polymers tested with incorporation of either just oxygen or oxygen and nitrogen functional groups, depending on the plasma.

The actual degree of substitution depended on polymer and plasma, but was in the order of 10–20% in UHMWPE and PTFE, and 25–50% for PEU and PDMS. The change in the surface from non-polar to polar groups would be expected to change the characteristics of the protein adsorption to the surface, considering the amphiphilic

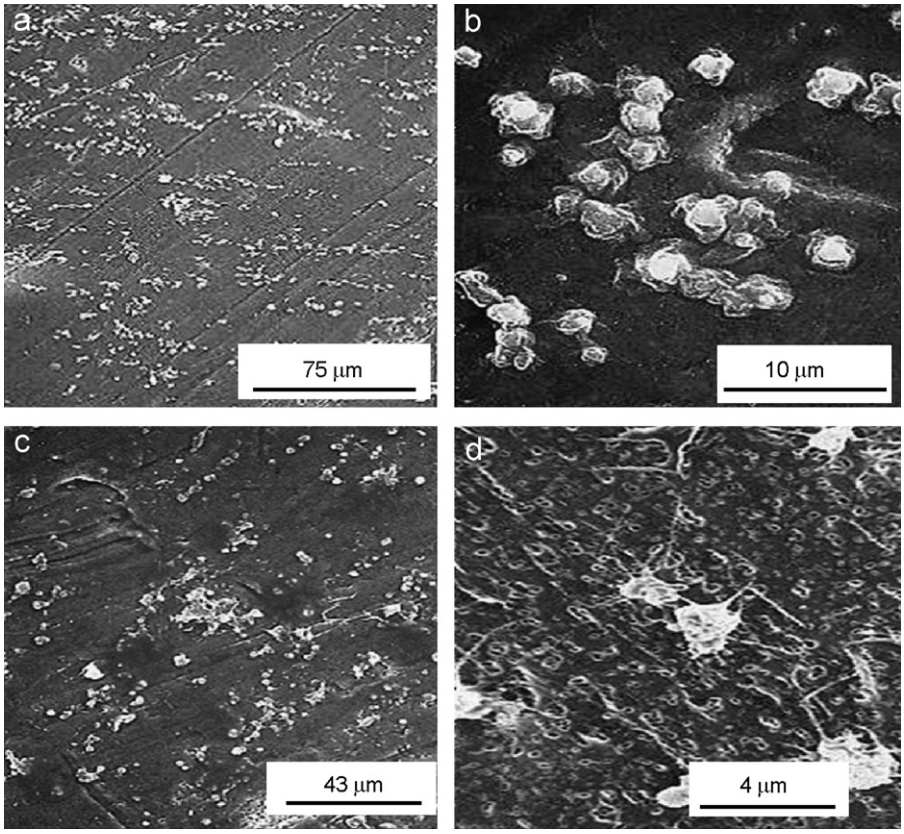

Fig. 6. SEM micrographs of platelets adhered onto (a, b) O_2 and (c, d) Ar plasma-treated PTFE.

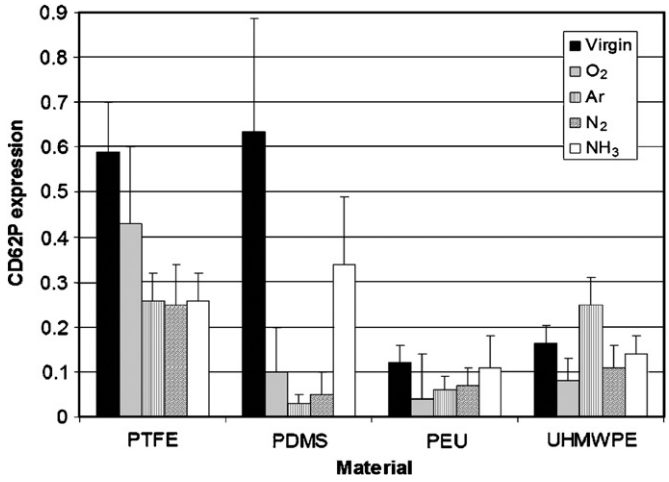

Fig. 7. CD62P expression on fluid-phase platelets after shearing of blood with untreated and plasma-treated materials. Mean ± SEM, $n = 8$, measured in arbitrary units of expression greater than resting platelets and where 95% of platelets stained with an isotype control antibody measure 1.0.

nature of proteins. In particular, it would be expected that the adsorbed proteins would undergo less conformational change due to their interaction with the more polar surfaces than on the untreated non-polar surfaces. However, increased surface water concentration has been shown to increase thrombogenicity [3] rather than reduce it. Although few rules of thumb exist, it is generally observed that increased surface hydroxyl concentration causes increased complement activation [8], whereas increased methylation results in reduced coagulation activation [29]. Additionally, increased surface oxygen concentration has been shown to reduce coagulation activation [30,31], whereas increasing surface negative charge is generally observed to increase surface activation [32,33].

If we consider UHMWPE and PTFE, plasma treatment of these surfaces resulted in a significant increase in wettability for all plasma gases. In all cases, therefore, there was a reduction in the C–H and C–F character, respectively, and an incorporation of polar functionalities of either or both oxygen and nitrogen. Analysis of the blood response to these materials demonstrates, however, significantly different responses. Significantly greater numbers of platelets were adhered to the UHMWPE surfaces than the PTFE surfaces. Fluid-phase CD62P expression was higher after blood contact with all PTFE surfaces compared to UHMWPE. Platelet aggregation index (PAI) was similar on untreated UHMWPE and PTFE but Ar, N and NH_3 treatment significantly reduced the PAI on PTFE but similar treatments had no affect on the PAI of UHMWPE. The microparticle (PMP) shedding index was significantly lower on all PTFE surfaces than the UHMWPE surfaces and, in particular, N_2 plasma treatment of UHMWPE caused a large increase in the PMP shedding index whereas the same treatment on PTFE had very little effect. In terms of the kaolin activation power

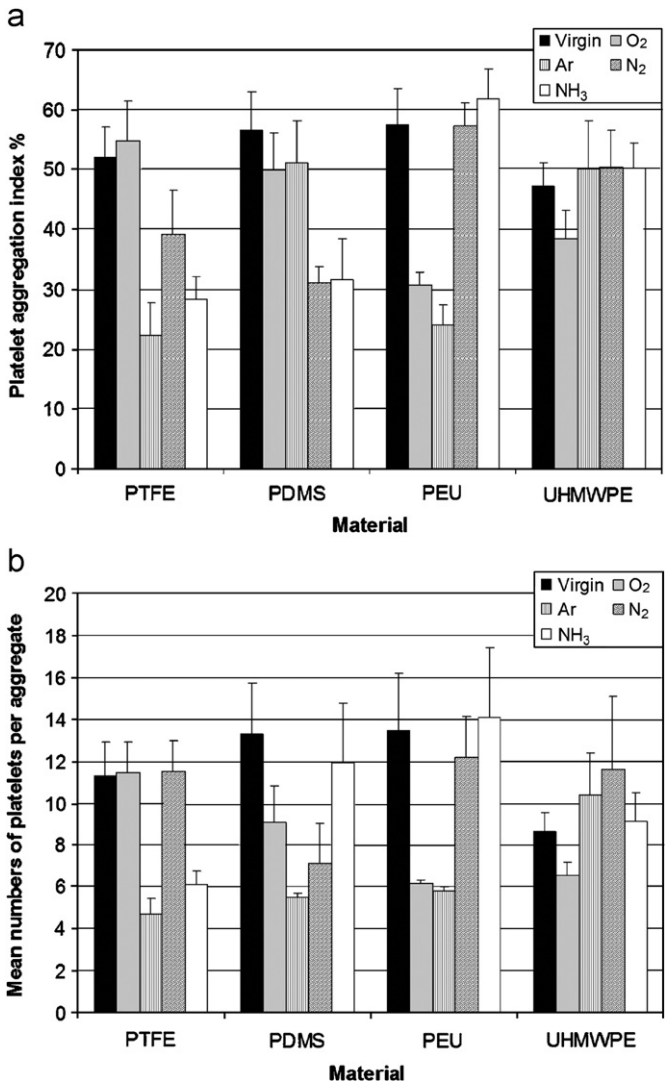

Fig. 8. (a) Platelet aggregation index and (b) mean platelet aggregate size (platelets per aggregate) after shearing with whole blood in contact with untreated and plasma-treated materials. Mean ± SEM, $n = 8$.

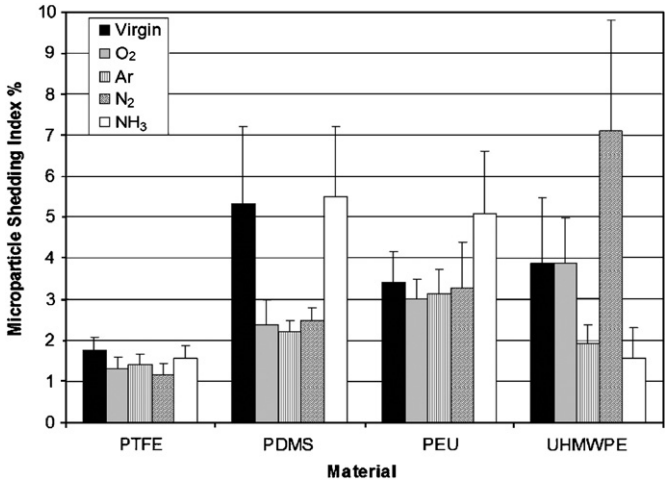

Fig. 9. Platelet microparticle shedding index after shearing of blood with untreated and plasma-treated materials. Mean ± SEM, $n = 8$.

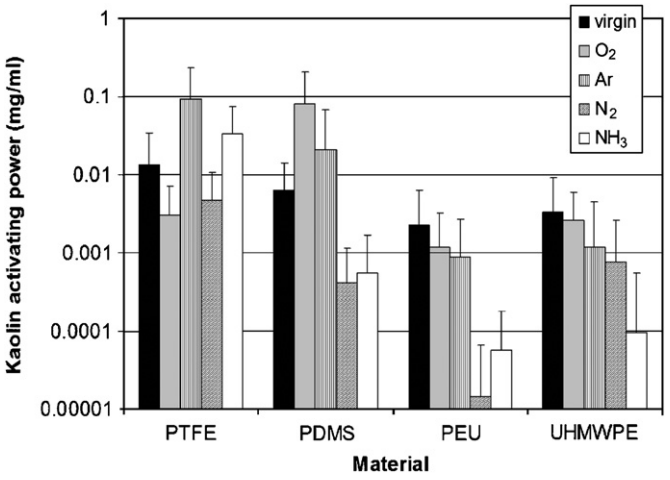

Fig. 10. Relative plasma clotting potential of untreated and plasma-treated materials as a proportion of that of 1000 mg l^{-1} kaolin. Mean ± SEM, $n = 6$.

(KAP), O_2 and N_2 plasma treatment of PTFE reduced the KAP whereas the Ar and NH_3 treatment increased it. However, for UHMWPE all treatments reduced the KAP although this was significant only for the NH_3 treatment.

It was observed in previous studies that both nitrogen and ammonia plasmas had the effect of dramatically reducing contact phase of the coagulation system on both polyurethane and silicone polymers [18,19]. In this study, it was true also of UHMWPE, especially for ammonia plasma. It was not, however, true for PTFE, although nitrogen plasma slightly reduced activation. As observed in other studies over the years, no correlation between activation of the coagulation system and activation or adhesion of platelets could be detected in this study.

Interestingly, there was also no qualitative, let alone quantitative similarity between the activation status of platelets in the fluid phase compared to those adhered onto surfaces. This does, however, equate with observations of the dynamic nature of platelet adhesion by Feuerstein and Kush [34], who observed that platelets adhere to thrombogenic materials only transiently in a flowing environment, but this is somewhat at odds with conclusions often drawn in relation to surface-bound platelets in other studies. This would imply that there may be many platelets within the fluid phase that became activated due to the transient interaction with the surface. Additionally, it would also be true that some materials may retain their platelets better than others, in a manner unrelated to their degree of thrombogenicity. Total platelet activation must, therefore, be considered a combination of contributions from fluid-phase and material-bound cells.

An explanation for the discrepancy in differences in haemocompatibility trends between surfaces for the same treatment could be the different reactivities of the plasmas to the different materials. The degree of functionalisation is similar on PDMS and PEU, but the reactions to blood follow different trends, for example platelet aggregation.

The same case could also be made for UHMWPE and PTFE, but the trends in reactions to these two treated polymers are also different. Similar rates of surface functionalisation have affected the nature of the adsorbed protein layer, and this is most likely due to heterogeneity in group substitution between materials, for example the microheterogeneity of crystalline and amorphous domains of PEU. It must be stressed that while similar chemical changes initially take place within diverse polymers, such as defluoriniation (in the case of PTFE) or dehydrogenation, chain scission and incorporation of oxygen or nitrogen-containing groups, further changes take place within air and water. Furthermore, surface relaxations and trapped reactive species will cause additional reactions to take place during contact with blood, with initial surface compositions evolving further.

One might expect platelet reactions to be expressed in similar proportions on similar surfaces. This does not seem to be the case, even when separated into fluid-phase and surface-phase compartments. This is particularly evident when one considers PMP shedding, CD62P expression, platelet aggregate formation and platelet adhesion. This may be because platelet aggregate formation is initiated when receptor GPIb associates with vWf in sheared blood, rather than the interaction of platelet $\alpha_{IIb}\beta_3$ with surface-bound, conformationally disturbed fibrinogen during platelet adhesion to a biomaterial. The role of the surface, therefore, is unclear in relation to the formation of platelet aggregates, and their activation status, but it has been observed in previous studies [22]. In this study, surface treatments were responsible for reducing platelet aggregate formation by half compared to a virgin material in approximately 40% of cases. Additionally, surface treatments reduced PMP shedding in more than 30% of the modifications. Essentially, PMP shedding and aggregate formation, both shear rate and temperature-dependant processes [21,23], have contributions from the surface-phase compartment. This is consistent with Feuerstein and Kush's [34] observations of transient platelet adhesion.

The reaction of a host to a blood-contacting device depends on the anatomical location and application of the device, and so the manner in which a polymer would be modified in order to reduce this impact would depend strongly on the relevance of the different parameters for the particular application (e.g. platelet activation in stenting). For small surfaces that are in high shear environments, the high rate of formation of activated species (platelets, PMPs, etc.) may be smaller than the platelet re-formation rate and so, while undesirable, be of little significance. The rate of small vessel occlusion due to platelet adhesion, activation and coagulation on indwelling tubes may be of much greater significance. However, the role of these different activated species in the aetiology of different device-related phenomena remains unclear.

However, we have demonstrated that some simple strategies exist for dramatically reducing certain unwelcome blood–material interactions, and that by careful matching of the plasma type with the biomaterial of choice for a particular application, it is possible to engineer a much more desirable blood response.

5. Conclusions

In conclusion, although different gas plasma treatments of a range of polymers can be shown to result in the incorporation of similar surface functional groups for each plasma type, there is no clear correlation between this incorporation and the fluid-phase and surface-phase reactions that result. However, our results demonstrate that there are some clear and relatively simple chemical strategies for dramatically reducing protein and cell activation events that are clearly implicated in the aetiology of disease states commonly observed after implantation of various blood-contacting devices.

Acknowledgements

The authors of this paper would like to thank Dr. Hans Griesser (CSIRO, Melbourne) for kindly providing XPS facilities for valence band spectra acquisition, Tim Steele and John Eccles of Millbrook Ltd. and Walter Weaver of BNFL without whose assistance the SSIMS studies would not have been possible. In addition, the EPSRC are acknowledged for providing a Ph.D. studentship for Darren Wilson and an Advanced Research Fellowship for Nicholas Rhodes. Parts of the research were performed as part of the Interdisciplinary Research Collaboration in Tissue Engineering for which funds were provided by the BBSRC, MRC and EPSRC.

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