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Chemiluminescent oxidative products generated by *in vitro* leukocyte–material interactions

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The effect of different polymeric materials on leukocyte adhesion and their oxidative responses was investigated. Two commercial polymers: polyethylene (PE) and silicone rubber (SR) and two laboratory-synthesized polyurethanes were examined. The polyurethanes studied consisted of a polytetramethylene oxide (PTMO)-based polyurethane (PEU-base) and a sulfonated polyurethane (PEU-SO3). *In vitro* polymorphonuclear leukocyte (PMN) adhesion and respiratory burst activity measurements were studied using a radiolabelling technique and a chemiluminescence (CL) assay, respectively. An increased number of adherent PMNs and increased cell spreading were found on PEU-SO3 compared to the PEU-base, SR and PE. The largest CL response was also found on PEU-SO3, whereas on the other hydrophobic polymers the response was smaller. Additionally, on the PEU-SO3 surface the CL response was sustained indicating more prolonged production of oxygen radicals by PMNs on this material. Upon stimulation with opsonized zymosan (OZ), the PMNs on PEU-SO3 showed the lowest CL response, indicating a decreased respiratory burst activity. The other hydrophobic surfaces, PE, SR and PEU-base, showed no significant differences among them in their CL response. The residual CL response was reduced by 30% for PEU-SO3 and by 10–15% for PE, SR and PEU-base compared to the control (luminometer polystyrene cuvette only, no disc). Upon addition of phorbol myristate acetate (PMA), all surfaces except SR showed a slightly reduced CL response. Along with PE, PEU-SO3 showed the lowest CL response. These results suggest that oxygen radicals are produced during early incubation of PMNs with biomaterials, and upon subsequent stimulation of these cells, fewer oxygen radicals are produced due to cellular exhaustion.

1. Introduction

Implanted medical devices induce various degrees of non-specific foreign body inflammatory responses which are critical to their integration and susceptibility to infection [1]. A major component of the inflammatory system consists of neutrophilic polymorphonuclear leukocytes (PMNs). These blood cells are capable of chemotaxis, adhesion, aggregation and phagocytosis in response to inflammatory stimuli [2, 3]. These leukocyte responses might also be expected to occur when blood contacts polymeric materials used in hemodialysis, cardiopulmonary bypass and vascular grafts. Biomaterials may also activate PMNs that will lead to the secretion of lysosomal proteolytic enzymes and high-energy oxygen species. Lysosomal enzymes have been shown to provoke an acute inflammatory reaction and tissue injury. Production of highly reactive oxygen intermediates such as superoxide anion (O_2^-), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH·) is also an integral part of the PMN bactericidal killing mechanism. The production of the oxidative products may also enhance the degradation of implanted biomedical materials.

The enhanced risk of bacterial infections in the vicinity of a foreign body, such as sutures and metallic or polymeric implants, has been known to occur during cardiovascular [4], orthopedic [5], plastic reconstructive [6], and general surgery [7]. The factors responsible for the enhanced risk of bacterial infection of foreign bodies are not well understood. Ineffectiveness of local host defence mechanisms against pyrogenic organisms has been suggested by many investigators [8].

Several studies have examined leukocyte activation by biomaterials. Most of these studies involved measuring accumulated concentrations of inflammatory products after certain periods [9, 10]. Kaplan and Piccolo [11, 12] developed a real-time method to monitor continuously the generation of oxidative products following cell–biomaterial interactions using chemiluminigenic probes *in vitro*.

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A study involving ceramic biomaterials noted that, in the absence of serum, $CaHPO_4$ powder stimulated an oxygen burst in PMNs indicating that the PMNs had been activated [13]. In the presence of polymethylmethacrylate (PMMA), suppression of the superoxide response during the phagocytic phase and the bacterial killing capacity of the monocyte-derived macrophages was observed [14]. PMNs have also been shown to release superoxide anions after incubation with polyurethane and Velcro pile (used in Jarvik 7 heart). Modulation of the superoxide secretion is also affected by the composition of polyurethane materials. Materials containing methylene diisocyanate (MDI) were found to suppress the inflammatory phase of the response, while those containing toluene diisocyanate (TDI) enhanced it [15]. A decreased phorbol myristate acetate (PMA)-elicited neutrophil respiratory burst on exposure to cobalt–chrome alloy and polystyrene *in vitro* for 3 h has been reported [16].

The decreased respiratory burst activity has been associated with defective antibacterial and antiviral phagocytic defence which in turn increases the risk for infection [17–19]. Giridhar *et al.* [20] observed that short-term exposure (5 min) of alveolar macrophages to PMMA beads resulted in an increased oxidative burst; however, 3 h exposure resulted in a decreased subsequent PMA-elicited chemiluminescence response for macrophages. Another study by Barth *et al.* [21] reported that alveolar macrophages had a high affinity for adherence to PMMA and elicited a high oxidative response, whereas titanium bound about half as many alveolar macrophages and no oxidative burst was elicited. They correlated these observations to the poor tissue compatibility of PMMA and comparatively satisfactory biocompatibility of titanium.

Oxidative burst elicited by the cells is usually preceded by the process of adhesion. Adhesion of leukocytes to artificial surfaces is an important phenomenon in the evaluation of biomaterials because the number of adherent leukocytes is often related to their inflammatory response after implantation [22]. Leukocyte adhesion to polymer surfaces is also known to occur during many types of processes involving blood–material interactions such as hemodialysis [23, 24], hemofiltration [25], cardiopulmonary bypass [26], artificial heart implantation [27] and nylon fibre filtration leukopheresis [28].

In the work presented here, the effect of polymeric materials on leukocyte adhesion and their oxidative response was investigated. Previous work by Grasel [29] has demonstrated that polyurethanes which are modified by grafting propyl sulfonate groups onto the urethane nitrogens have extremely good blood contacting properties in a canine *ex vivo* shunt model. These ionomers in the dry state have higher tensile strength and modulus compared to the underivatized polymer due to the clustering of ionic groups. Upon hydration the ionomers swell considerably forming anionic hydrogels [30].

In these studies, the sulfonated polyurethane was laboratory synthesized. Propyl sulfonate groups were substituted for 17 mol % of the N–H groups of the underivatized base polyurethane. Two commercial polymers, polyethylene (PE) and silicone rubber (SR), were also examined and used as control hydrophobic surfaces. The surface of these materials has been characterized using static and dynamic contact angle measurements and X-ray photoelectron spectroscopy (XPS) [31]. Polymorphonuclear leukocytes (PMNs) were chosen to investigate leukocyte adhesion and oxidative burst activity on these polymer surfaces. *In vitro* leukocyte adhesion measurements were carried out by incubating the polymers with purified PMNs for 5, 30 and 60 min under static conditions. To quantify the number of adherent PMNs on the polymers, radiolabelling was utilized. The amount of lactate dehydrogenase released into the extracellular medium was used as an indicator for cell lysis. The PMN respiratory burst activity was studied using a real time *in vitro* chemiluminescence assay.

2. Materials and methods

2.1. Materials

The base polyurethane (PEU-base) was obtained from Becton Dickinson Polymer Research (Sandy, UT, USA). The PEU-base contains a hard segment of 55 wt % of methylene bis(p-phenyl diisocyanate) (MDI) and 1,4-butanediol (BD). Polytetramethylene oxide (PTMO) of 1000 M_w was used as the soft segment. Commercial polyethylene tubing/film (PE) (Intramedic, Clay Adams, Parsippany, NJ, 0.125 inch inside diameter) and silicone rubber tubing/film (SR) (Silastic, Medical Grade, Dow Corning, Midland, MI, 0.125 inch inside diameter, 0.25 inch outside diameter) were used as received. The PEU-base was extracted for two days using toluene in a Soxhlet extractor to remove low molecular weight impurities. The base polyurethane was dissolved in N,N-dimethylacetamide (DMAc) and then derivatized by a bimolecular nucleophilic displacement of the urethane hydrogen using NaH, followed by a ring-opening reaction with γ-1,3-propane sultone [29]. Before analysis, the sulfonated polyurethane was subjected to Soxhlet extraction using toluene for 48 h in an effort to remove contaminants, oligomeric species, and residual propane sultone if present. A sulfonated polyurethane (PEU-SO3) with 17% of its urethane hydrogen substituted with propyl sulfonate groups was used in this study.

2.2. Material preparation

Polymer-coated tubing (0.125 inch inside diameter) was prepared in order to study *in vitro* cell adhesion. The polyurethanes were coated from a 4% solution in DMAc to the inside of oxidized Intramedic PE tubing using the procedure described by Lelah *et al.* [32]. The tubing was oxidized with a chromic acid solution (chromerge) at 70 °C for 30 min, rinsed thoroughly with dilute nitric acid and deionized water, and then drained and dried in a nitrogen gas stream for 7 days followed by drying in a vacuum tube at 50 °C for 2 days.

For chemiluminescence experiments, the polyurethanes were solvent-cast from 4% polymer

solutions in DMAc onto 8 mm diameter glass coverslips. Before casting, the coverslips were first cleaned in Chromerge for 30 min, then washed with two changes of deionized water for 30 min each, methanol for 30 min, and then dried in a vacuum oven overnight.

2.3. Preparation of polymorphonuclear leukocytes (PMNs)

Human PMNs were prepared using a double density gradient centrifugation technique [33]. Peripheral blood from healthy adult donors was collected into a syringe containing EDTA anticoagulant. In a 50 ml polystyrene centrifuge tube, 5 ml of light Ficoll (density = 1.077 g/ml, Sigma Chemical Co., St. Louis, MO, USA) was layered over 13 ml of heavy Ficoll (density = 1.118 g/ml, Sigma Chemical Co., St. Louis, MO, USA). 20–25 ml of whole blood was then carefully layered on top of the Ficoll gradient and centrifuged at 1300*g* for 30 min at room temperature. The PMN layer was then transferred to a clean 50 ml tube, washed with Hank's Balanced Salt solution (HBSS, Gibco, Grand Island, NY), and centrifuged at 850*g* for 10 min. At the end of centrifugation, 10 ml of lysing buffer (0.155 M ammonium chloride, 0.010 M sodium bicarbonate, 0.0003 M EDTA, pH = 7.4) was added to the cell pellet and quickly centrifuged at 500*g* for 10 min. The resulting cell pellet was then resuspended in HBSS, and centrifuged at 500*g* for 10 min at room temperature. This procedure was repeated twice. After the last wash, the cell pellet was resuspended in 1 ml of autologous platelet-free plasma. The viability and the purity of the cells were examined using optical microscopy. The purity of the cell suspension was determined using Wright's stain and showed that 95–98% of the cells were PMNs. The viability of the cells was always greater than 95% as determined by trypan blue exclusion.

2.4. Radiolabelling of PMNs

The PMNs were radiolabelled with ^{111}In-tropolone according to the method of Dewanjee [34]. ^{111}In-tropolone was mixed with the cell suspension and incubated for 30 min at room temperature. This method of labelling results in binding of ^{111}In to the cell membrane and organelles and consequently there is very little release due to sublytic damage [34]. This method resulted in a labelling efficiency of 80–95%. In addition, this method showed no effect on cell viability [34].

2.5. Leukocyte adhesion assay

Leukocyte adhesion experiments were carried out using 1.5 inch segments of the test polymer tubings connected together in random order. 0.5 inch silicone rubber tubing was used as sleeves to hold the sections together and three-way stopcocks were attached at both ends. Commercial PE and SR tubing was used to provide model hydrophobic surfaces and to serve as controls. Prior to the adhesion studies, the tubing series was washed with 100 tubing volumes of double distilled deionized water and then equilibrated with HBSS overnight. Previous studies demonstrated that the presence of more than one polymer material together in a tubing series does not affect the adhesion results.

To perform the leukocyte adhesion experiments, a radiolabelled-cell suspension (1.0×10^6 PMNs/ml in HBSS) was infused into the tubing with a syringe via the three-way stopcock. The stopcock was used to avoid the formation of an air–water interface. A total cell suspension volume of three times the tubing volume was used to introduce the cells. This volume has been shown to substantially remove the initial buffer [35]. The PMN suspension was incubated in the polymer tubing for time periods of 5, 30, and 60 min at room temperature. At the end of the incubation period, the tubing series was flushed at 100 ml/min with 20 tubing volumes of buffer to remove the non-adherent cells, and fixed with a 2% glutaraldehyde solution. Each test section was then subdivided into sections for gamma counting and preparation for scanning electron microscopic examination. Typically 1 inch specimens were cut from each and counted in an automated gamma counter (Beckman 5500 or Packard 5360) to determine the amount of adherent radioactivity.

2.6. Scanning electron microscopy (SEM)

After fixation with 2% glutaraldehyde, polymer tubing samples were serially dehydrated in ethanol/water solutions, dried using the critical point method, mounted, and sputter-coated with gold for examination in a JEOL-JSM 35C SEM using an accelerating voltage of 15 kV.

2.7. Membrane lysis assay

As an indicator of membrane damage and cell lysis, a lactate dehydrogenase (LDH) assay which was obtained from Sigma (Sigma, St. Louis, MO) was used.

2.8. Chemiluminescence (CL) assay

The activation of PMNs by phagocytosis has been shown to be associated with the production of light that can be measured on a liquid scintillation counter or a luminometer [36]. The natural light or native chemiluminescence produced by the cells during the phagocytic process can be amplified by adding a chemical known as Luminol (5-amino-2,3-dihydro-1,4 phthalazinedione) to make the methodology a very sensitive technique for evaluating the phagocytic function.

The chemiluminescence assays were carried out after placing the 8 mm polymer coated coverslip into the bottom of 4 ml polystyrene tubes. 1×10^6 PMNs in HBSS-HSA (2 mg/ml) was then added to the tube and allowed to interact with the polymer for 1–2 min. Luminol (2×10^5 M) and HBSS buffer was then added to the tube to make a final volume equal to 1 ml. CL

was measured continuously for 30 min using a Los Alamos Diagnostics Model 633 Chemiluminometer, equipped with a Model 1291 dispenser for dispensing activator. The stimulants used included opsonized zymosan (1 mg/ml) and phorbol 12-myristate 13-acetate (PMA, 1 μg/ml). The temperature of the test was 37 °C. Counts were done using the "integrate" mode with an integration time of 0.5 s. Units are mVs/0.5 s (millivolt-second per half second), i.e. the area under the curve for each counting interval.

3. Results and discussion

3.1. *In vitro* adhesion of PMNs to polymers under static conditions

Figs 1–4 show the results of the leukocyte adhesion experiments. Fig. 1 shows that the number of adherent PMNs on sulfonated polyurethane (PEU-SO3) is significantly higher at the 99% confidence level than the corresponding values for PEU-base. The degree of adhesion for the two more hydrophobic polymers, PE and SR, was comparable to that of PEU-base. The most hydrophobic of the two, SR, showed a slightly lower value of adhesion at the 5 min time point. The number of adherent cells on all surfaces increased significantly with time up to 30 min and levelled off thereafter.

Scanning electron micrographs of adherent PMNs to these surfaces revealed interesting differences in the leukocyte-surface interaction that depended not only on sulfonate group content but also on the degree of hydrophobicity of the polymers. In agreement with Fig. 1, Figs 2 and 3 show higher density of adherent PMNs on the sulfonated polyurethane (Figs 2b and 3b) than on PEU-base (Figs 2a and 3a) at 5 and 60 min. In addition, adherent PMNs on the sulfonated polyurethane appeared to have a higher degree of spreading. At 60 min, more than 85% of adherent PMNs on the sulfonated polyurethane (Fig. 3b) had extended their cytoplasmic membrane and spread

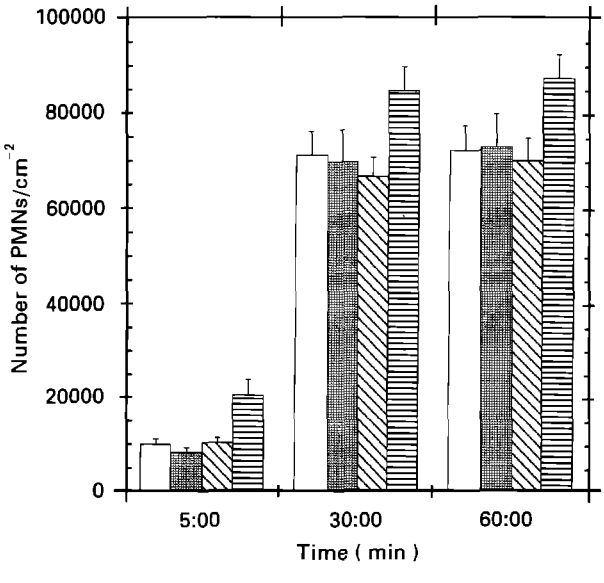
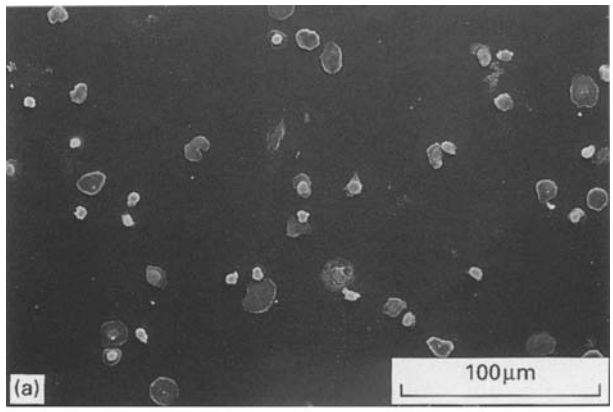


Figure 1 In vitro polymorphonuclear leukocyte (PMN) adhesion to polymers: □ PE; ▦ SR; ▨ PEU-base; ▤ PEU-SO3.



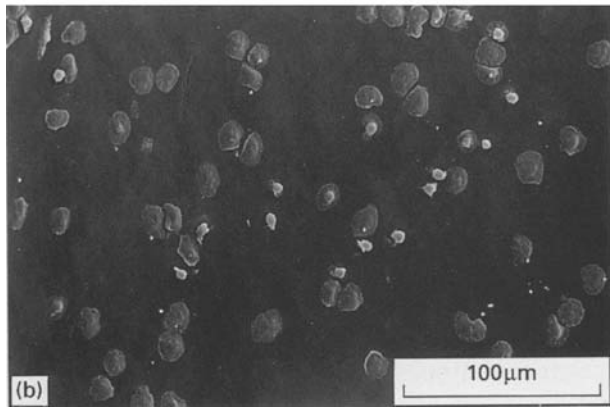
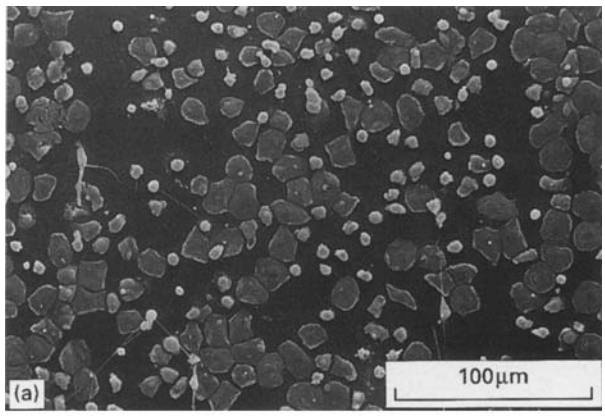


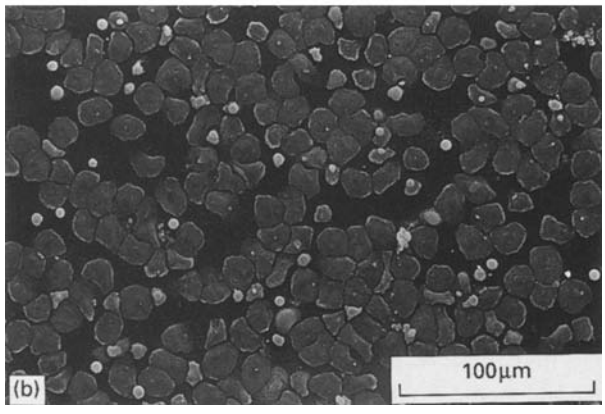
Figure 2 Scanning electron micrographs of adherent PMNs after 5 min adhesion time on (a) PEU-base; (b) PEU-SO3.

over the polymer surface, while more than 40% of adherent cells on PEU-base (Fig. 3a) still maintained their original spherical shape. SEM micrographs for 30 min adhesion time (not shown) were similar to the 60 min time point.

SEM micrographs for PE and SR are shown in Fig. 4. As seen in Fig. 4, the number of adherent PMNs on SR at 5 min was lower than the corresponding value for PE, and the density of adherent PMNs on PE is similar to PEU-base. An interesting morphology difference between adherent neutrophils on these two hydrophobic materials was observed. Closer examination of the cells revealed significant cell spreading on PE surfaces at both 5 and 60 min. Figs 4b and 4d show the morphology of adherent cells on SR. Most of these cells appeared to be round and had undergone membrane ruffling. No significant cell spreading on SR was observed.

The relatively high number of PMNs adhering to the sulfonated polyurethane compared to PEU-base was unexpected. Since leukocytes bear a negative charge that results from their lipoprotein membrane structure [37], electrostatic forces may be expected to influence cell adhesion. It has been reported that negatively charged groups such as carboxylate and sulfonate groups reduced cell adhesion [38, 29]. Another factor that may influence leukocyte adhesion to solid surfaces is surface free energy. Neumann *et al.* [39, 37] showed enhanced leukocyte adhesion to surfaces with higher surface free energy or increased wettability.





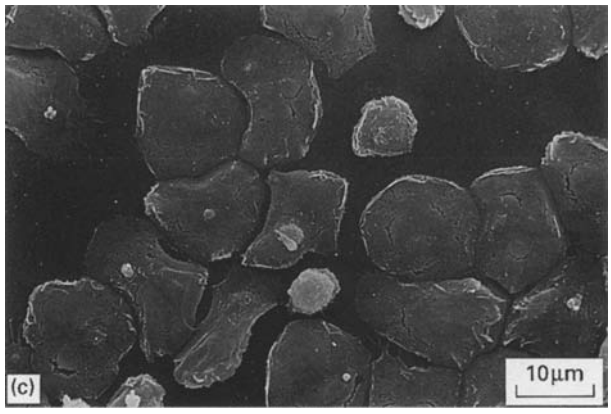
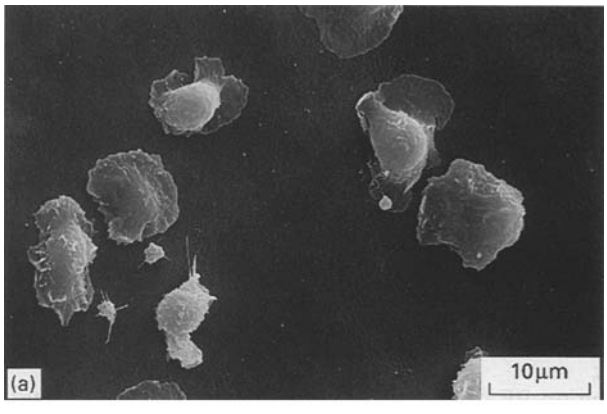


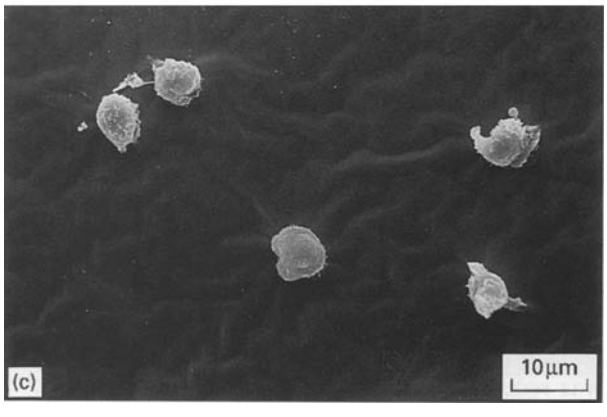
Figure 3 Scanning electron micrographs of adherent PMNs after 60 min adhesion time on (a) PEU-base; (b) PEU-SO3 (390 ×); (c) PEU-SO3 (1500 ×).

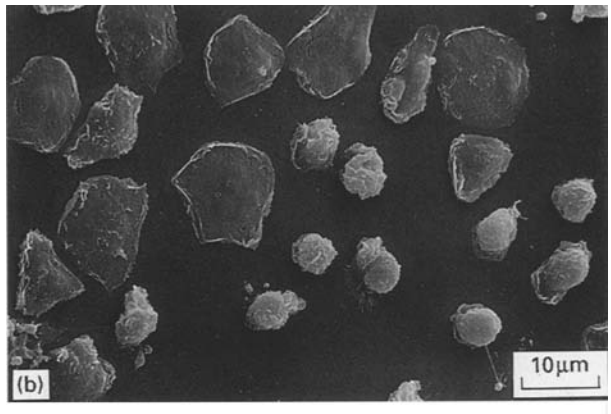
Such a relation would explain the higher number of adherent PMNs on the sulfonated polyurethane compared to the more hydrophobic polymers: PE, SR and PEU-base. However, the higher number of adherent PMNs on SR relative to PEU-base cannot be explained in terms of surface free energy arguments. The enhanced PMN adhesion to the sulfonated polyurethane may also be related to the nature of the sulfonate functional groups. Unpublished results from Proctor *et al.* [40] also showed enhanced adhesiveness of PMNs to glass in the presence of heparin.

3.2. Membrane lysis assay

The absence of measurable lactic dehydrogenase activity for all polymers tested indicated that there was







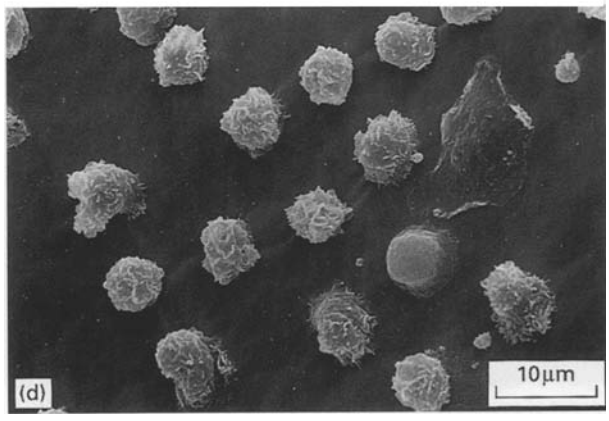


Figure 4 Scanning electron micrographs of PMNs adherent to PE and SR after 5 and 60 min: (a) PE, 5 min; (b) PE, 60 min; (c) SR, 5 min; (d) SR, 60 min.

TABLE I Statistical examination of chemiluminescence response data using two-sided *t*-test

Materials being compared	Material only $t \leqslant 10$ min (Fig. 5)	Material + OZ (Fig. 6)	Material + PMA (Fig. 7)	Material and PMA (Fig. 8)
No disc PE	−	+ +	+ + +	−
No disc SR	+	+ +	−	−
No disc PEU-base	+ + +	+ +	+	−
No disc PEU-SO3	+ + +	+ + +	+ + +	−
PEU-base PEU-SO3	+ + +	+ +	+ +	−

+ = null hypothesis (that means are identical) may be rejected at 90% level of confidence
+ + = null hypothesis may be rejected at 95% level of confidence
+ + + = null hypothesis may be rejected at 99% level of confidence
− = null hypothesis may not be rejected at confidence interval level $\geqslant 90\%$

no cell lysis occurring as a result of PMN interactions with the polymers.

3.3. Effect of polymeric materials on PMN oxidative burst activity

The results for the PMN oxidative metabolism analysis by continuous measurement of the PMN chemiluminescence (CL) response are shown in Figs 5–8 and Table I. Fig. 5 shows representative curves of the CL response as PMNs interacted with the surfaces for a period of 30 min. Table I contains the results of two-sided *t*-tests that were performed for selected pairs of surfaces under different conditions. The CL response elicited by the biomaterials in the absence of stimulants peaks within 3–6 min. The largest CL response was found on PEU-SO3, whereas on other hydrophobic polymers the response was smaller. Additionally, on the PEU-SO3 surface the CL response was more sustained, indicating more prolonged production of oxygen radicals in PMNs on this material. Table I shows that the CL response on PEU-SO3 is significantly higher at the 99% confidence level than the corresponding values for PEU-base and control. The higher CL response on PEU-SO3 correlated with the increased PMN adhesion and spreading. Other workers have also shown that increased adherence and spreading of PMN to non-phagocytosable surfaces led to increased activation of oxygen metabolism and to defective bactericidal activity [41, 42]. Another observation by Giridhar *et al.* [43] found that laboratory prepared PMMA particles that adhered to macrophages elicited an oxidative burst, while commercial PMMA had no significant adherent macrophages and a minimal CL response.

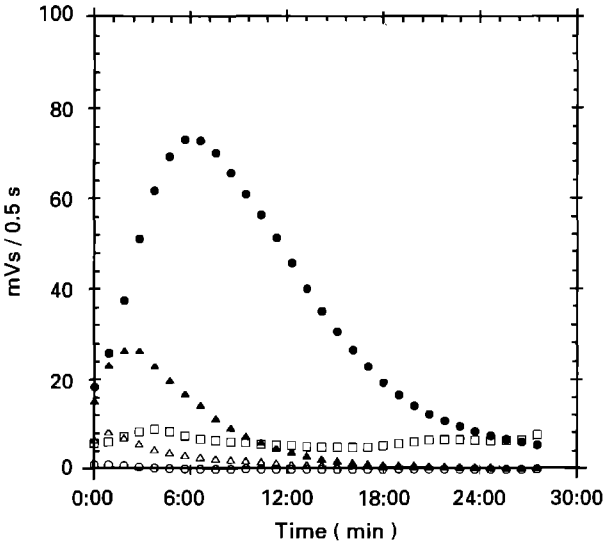


Figure 5 Chemiluminescence (CL) (average of six experiments) response of polymorphonuclear leukocytes (PMNs) to PE, SR, PEU-base and PEU-SO3. Reaction mixture included: polymer-coated coverslip, 1×10^6 PMNs in 1 ml HBSS-albumin and luminol (2×10^{-5} M): ○ no disc; △ PE; □ SR; ▲ PEU-base; ● PEU-SO3.

3.4. Residual oxidative burst activity

To determine if the initial acute oxidative burst elicited by the biomaterials would exhaust the PMN response to a subsequent stimulation/challenge, stimulants were added. In the measurements of the residual oxidative burst activity of the PMNs, a soluble activator, phorbol 12-myristate 13-acetate (PMA), and a non-soluble activator, opsonized zymosan (OZ) were used to stimulate the PMNs. Figs 6 and 7 and Table I show the CL response of PMNs on surfaces upon addition of opsonized zymosan and PMA, respectively. The stimulants were added to the reaction mixture after the PMNs were allowed to interact with the surfaces for 30 min. All surfaces showed significantly lower response than the negative control at 95% confidence level. Upon stimulation with opsonized zymosan, the PMNs on PEU-SO3 showed the lowest CL response indicating a decreased respiratory burst activity. The other hydrophobic surfaces: PE, SR and PEU-base, showed no significant differences in their CL response. The residual CL response was reduced by 30% for PEU-SO3 and by 10–15% for PE, SR and PEU-base, compared to the control. Upon addition of PMA, all surfaces except SR showed a slightly reduced CL response. Along with PE,

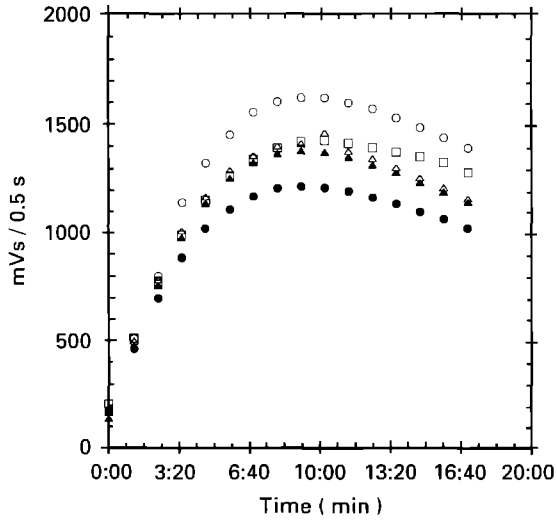


Figure 6 Chemiluminescence (CL) (average of six experiments) response of polymorphonuclear leukocytes (PMNs) to PE, SR, PEU-base and PEU-SO3 upon addition of opsonized zymosan. Reaction mixture included: polymer-coated coverslip, 1×10^6 PMNs in 1 ml HBSS-albumin, luminol (2×10^{-5} M) and opsonized zymosan (1 μg/ml): ○ no disc; △ PE; □ SR; ▲ PEU-base; ● PEU-SO3.

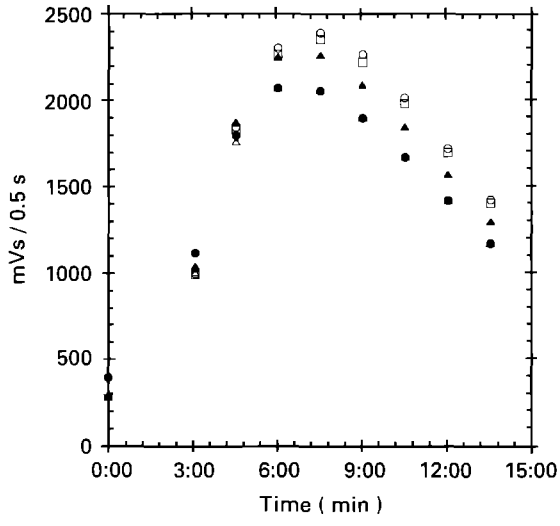


Figure 7 Chemiluminescence (CL) (average of six experiments) response of polymorphonuclear leukocytes (PMNs) to PE, SR, PEU-base and PEU-SO3 upon addition of PMA. Reaction mixture included: polymer-coated coverslip, 1×10^6 PMNs in 1 ml HBSS-albumin, luminol (2×10^{-5} M) and PMA (1 μg/ml): ○ no disc; △ PE; □ SR; ▲ PEU-base; ● PEU-SO3.

PEU-SO3 showed the lowest CL response. As expected, when the stimulant was added to the reaction mixture before the PMNs were allowed to interact with the polymers, there was no significant reduction in the CL responses, as seen in Fig. 8 and Table I. These results indicate that incubation of PMNs with polymeric materials reduced the subsequent (OZ)-elicited CL response. Except for SR, incubation of PMNs with the polymers studied also reduced the subsequent PMA-elicited CL response.

These results suggest that the *in vitro* interaction of PMNs with a non-phagocytosable biomaterial leads to partial exhaustion of PMN function, which may reduce PMN capability to mount a respiratory burst upon further stimulation. Sulfonate groups on polyurethane seemed to induce a higher oxidative burst activity compared to the underivatized polymers which subsequently leads to a decreased residual oxidative burst activity. The results obtained also confirm the earlier studies by other groups [16] who found that the more PMNs that adhere to polymers, the higher the CL response level. These observations indicate that PMN adherence to non-phagocytosable biomaterials may be needed for the oxidative burst activity. Elicitation of a CL response was also dependent on the different types of non-phagocytosable materials. The more hydrophobic materials, PE and SR, generated lower CL responses. The more hydrophilic polymers, PEU-base and PEU-SO3 generated significantly higher responses. This indicated that the chemistry of the polymeric materials plays a role in the generation of the PMN oxidative response.

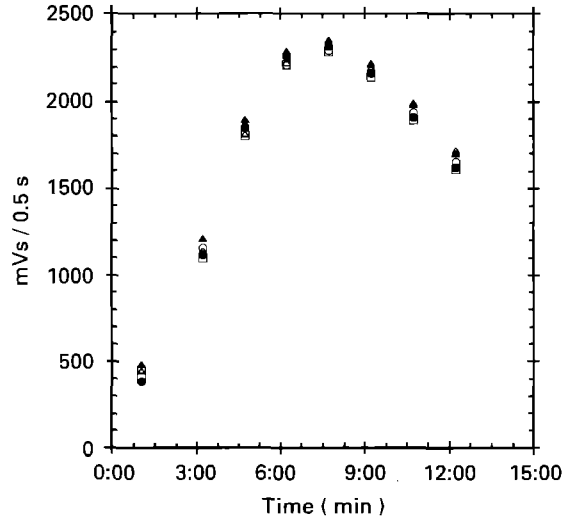


Figure 8 Chemiluminescence (CL) (average of six experiments) response of polymorphonuclear leukocytes (PMNs) to PE, SR, PEU-base and PEU-SO3 with simultaneous addition of PMA. Reaction mixture included: polymer-coated coverslip, 1×10^6 PMNs in 1 ml HBSS-albumin, luminol (2×10^{-5} M) and PMA (1 μg/ml): ○ no disc; △ PE; □ SR; ▲ PEU-base; ● PEU-SO3.

4. Conclusions

Our results indicate that short-term exposure of PMNs to polymeric materials can generate a respiratory burst. This exposure diminishes the PMNs' subsequent OZ- and PMA-elicited CL responses. It is also suggested that the capacity of PMNs to adhere and spread on biomaterials determine the potential of a non-phagocytosable biomaterial to elicit an oxidative burst. This property might influence the propensity of a biomaterial to be colonized by bacteria, since exhausted leukocytes would have diminished ability to destroy infecting cells.

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