JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE 8 (1997) 239–245

Screening of biomedical polymer biocompatibility in NMRI-mice peritoneal cavity

A comparison between ultra-high-molecular-weight polyethylene (UHMW-PE) and polyethyleneterephthalate (PET)

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The peritoneal resident cell population is influenced by various inflammatory and immunogenic stimuli. The influence of intraperitoneal application of polyethyleneterephthalate (PET) (group A) and ultra-high-molecular-weight polyethylene (UHMW-PE) (group B) powders on peritoneal cell count and macrophage activity was investigated. Powders were tested to mimic wear particles from solid implant devices as these particles often cause chronic granulomatous inflammation. The results were compared with the inflammatory response following an abdominal midline incision (group C) and untreated animals (group D). On days 1, 7, 14 and 30 peritoneal cells were quantified and the number of active macrophages was assessed. Groups A and C mice showed a significant loss of macrophages in the peritoneal lavage at day 1 but this returned to normal values (group D) on day 7. In contrast, group B animals remained at low peritoneal cell counts but showed the highest number of active macrophages. Only in this latter group was adhesion formation and granulomatous clustering of polymer powder observed. Applying the parameters macrophage count and the number of active macrophages it can be concluded that PET elicits a weaker inflammatory reaction than UHMW-PE in mice peritoneal cavity. Thus this animal model may be used as a screening test for biomedical materials, especially their wear products.

1. Introduction

Implanted biomaterials are subjected to mechanical, oxidative and enzymatical stress which can change their physicochemical properties and result in the liberation of wear products. These particles differ in their biocompatibility when compared with the solid implant [1]. There is strong evidence that particle size is inversely correlated to the strength of foreign body reaction [1, 2]. Whether wear particles impair biocompatibility of biomaterials by increasing total surface area or by a higher degree of mechanical tissue injury is not known. *In vivo* implantation tests show a higher degree of inflammation at uneven implant sites such as corners and edges compared with smooth surface areas (unpublished own data [3]). Thus the *in vivo* behaviour of wear particles has to be evaluated before judging a biomedical material as biocompatible. For example, ultra-high-molecular-weight polyethylene is known for its good biocompatibility. It is a reference material and other candidate polymers are compared with it. But these good features are restricted to solid UHMW-PE implants with smooth surfaces. On the other hand UHMW-PE in powder form is a potent inflammatory agent [4]. For some years, total hip endoprosthesis has utilized an implanted UHMW-PE hip cup because of the good resistance to stress cracking and abrasion of this material [5, 6]. Nevertheless, over a 5-year period after implantation one millimetre of the surface is abraded [7]. There is strong evidence that these UHMW-PE-wear particles can cause a chronic aseptic granulomatous inflammatory response leading to the loss of implant function [5]. Thus biomedical material testing has to examine not only solid implants but also

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their particulate samples to mimic the effects of wear products [6].

The peritoneal cavity is a well known source of macrophages and polymorphonuclear cells for immunological, inflammatory studies [8]. It has also been used to perform biomedical material testing [9]. The composition and number of peritoneal resident cells can be influenced by various inflammatory or immunogenic stimuli [10–12].

Therefore the aim of the present study is to compare the peritoneal cell counts after intraperitoneal application of polymer powders. The parameters tested are the composition of the peritoneal cell population and the activity of macrophages, indicated by their capacity to ingest china ink particles.

With a one-month observation time this model may be considered as a screening method to assess the degree of biocompatibility of biomedical materials, especially of their wear products.

2. Materials and methods

2.1. The polymer powders

2.1.1. Preparation of the polymer powders

Ultra-high-molecular-weight polyethylene (UHMW-PE, RCH 1000 Chirulene^{R}, Vertriebsgesellschaft für Technische Produkte mbH, Oberhausen, Germany) was available as cylindrical rods with a diameter of 2 cm. These rods were cut in pieces of 1 cm length. Subsequently, they were milled with the ultracentrifugal mill SM1 (RETSCH GmbH, Haan, Germany). These particles were finely ground with the ultracentrifugal mill ZM 1 (RETSCH). This last step was repeated with ring sieves of different pore sizes (first 2 mm, then 0.5 mm). Polyethyleneterephthalate (PET, PET 39 RS, Dacron^{R}, HOECHST AG, Frankfurt am Main, Germany) available as granules was directly treated with the mill ZM 1. To prevent the polymers from heating and the subsequent changes in their chemical surface properties, the polymers were cooled with liquid nitrogen to about 78 K before and during grinding. Applying a centrifugal dust classifier (licence BAHCO-Sweden, Neu GmbH, Übach-Palenberg, Germany) a fine powder fraction was separated. The powders were packed, gas sterilized with ethylene oxide and allowed to aerate for at least one week [13].

2.1.2. Analysis of particle sizes

First the particle morphology and size was assessed by a semi-automatic procedure using a digitizer board, Leitz-microscope and a personal computer. UHMW-PE particles had an average diameter of 68 μm ($n = 100$, standard deviation $+/-30$ μm) and PET 64 μm ($n = 100$, $+/-27$ μm) (Figs 1 and 2). The particles of the two powders were similar in morphology: a round, irregular and polygonal shape. In the PET-powder 12% of the particles were fibres of 15 to 20 μm width and an average length 374 μm. The fibre length varied between 19 and 1240 μm (Fig. 2). Analysis of particle size distribution in these fine powders was performed with a laseroptical system (Particle Sizer^{R} 2600 C, Malvern Instruments, Spring Lane South, UK). The powders were suspended in 70% ethanol. Each particle scatters precise quantities of laser light through precise angles related to their size (laser Fraunhofer diffraction). Particle size distribution is computed from this data. The size distribution of the polymer powders examined in this study are presented in Fig. 3: 90% of particles were smaller than 91.0 μm in the PET-powder and smaller than 93.3 μm in the UHMW-PE-powder (Fig. 3). The software of the particle sizer allows one to compute various average diameters concerning the distributions of particle number, length, surface or volume. These different diameters for PET and UHMW-PE are compared with those of monodisperse latex spheres (19 μm) (Table I). While for the spherical latex particles the average diameters are the same, both PET and UHMW-PE show different diameters. This proves the polydisperse nature of the powder particles. Comparing diameters of the same distribution it can be concluded that both polymer powders share common geometric features.

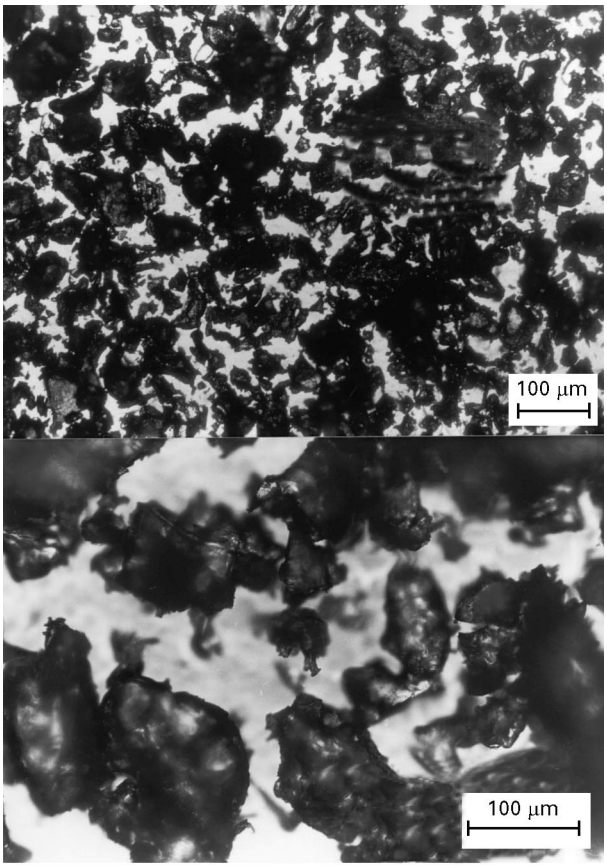

Figure 1 Light micrographs of the UHMW-PE powder fraction obtained after treatment with a centrifugal dust classifier. The particles are of round, irregular and polygonal shape.

2.2. Animals

Mice, Ico: NMRI-Han, female, IFFA-CREDO (France), age 38–42 days, weight 23–26 g, free of specific pathogens. The animals were housed under conventional conditions and allowed two weeks to adapt to the laboratory environment before the start of the experiment. At this time the animals reached an average weight of 40 g.

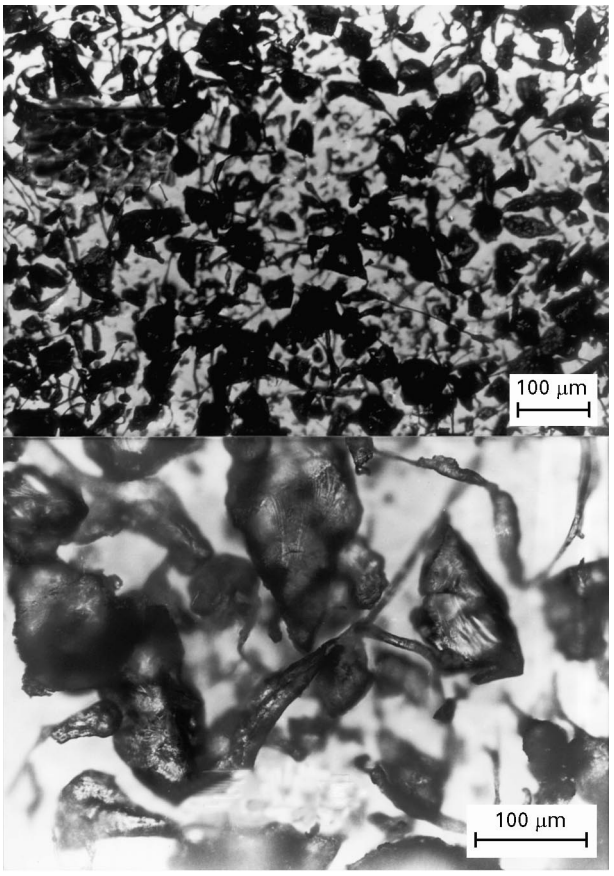

Figure 2 Light micrographs of the PET powder fraction obtained applying a centrifugal dust classifier. The particles are of round, irregular and polygonal shape and are similar to the UHMW-PE particles. Some particles are fibre-shaped.

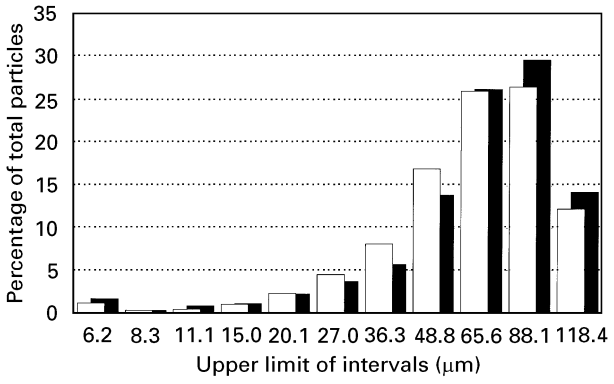

Figure 3 Size distribution of PET (□) and UHMW-PE (■) particles. The percentage of particles in 11 intervals is shown. The values on the x-axis give the upper limit of size intervals.

2.3. Experimental protocol

Animals were divided into four groups. Group A was administered 40 mg of PET powder intraperitoneally and group B, 40 mg of UHMW-PE-powder. Group C animals were subjected to a sham operation to evaluate the impact of the surgical trauma and wound occlusion material (Catgut and Histoacryl blue^{R}). Group D was composed of 13 untreated control animals. Groups A, B and C contained 20 animals each.

TABLE I Average diameters calculated from volume, surface, length or number distribution. While, for the spherical latex particles the average diameters are the same, both PET and UHMW-PE show different diameters. This proves the polydisperse nature of the polymer particles. Comparing diameters of the same distribution it can be concluded that both polymer powders share common geometric features.

Diameters calculated from distributions of	UHMW-PE particles (μm)	PET particles (μm)	Latex spheres (μm)
volume	61.27	58.87	19.31
surface	38.53	40.22	19.02
length	10.20	13.96	18.75
number	3.97	5.33	18.48

2.4. Experimental procedure

Animals were anesthetized by ether inhalation. After removal of hair and disinfection of the skin, the peritoneal cavity was opened in the linea alba of musculus rectus abdominis by a small incision. In group C animals no polymer powder was applied and the peritoneum and abdominal muscle were closed with one Catgut suture and the skin with Histoacryl blue^{R}. Group A and group B were administered 40 mg of PET or UHMW-PE, respectively, and then the opening was closed as described above. On day 1, 7, 14, 30 after this surgical treatment five animals of each group A, B or C were sacrificed by ether overdose. The peritoneal cavity was opened above the operation scar to avoid artificial perforation of the intestine and then rinsed three times with 5 ml of tissue culture medium TCM 199 with EDTA as an anticoagulant. Cells were counted in a counting chamber, after methylene blue staining. The number of cells counted was multiplied by the number of millilitres of peritoneal lavage fluid obtained from each mouse. The peritoneal fluid was centrifugated at 1200 rpm for 10 min, the supernatant was poured away and TCM 199 was added to yield 2×10^6 cells/ml. This cell suspension was given in a tube. This tube was incubated for one hour with green china ink (Faber Castell, Germany). Then the number of active macrophages was assessed by counting those macrophages having phagocytosed green ink particles. A mesenteric smear was examined to exclude infection.

2.5. Evaluation

After staining with May-Grünwald-Giemsa the cell types were qualified as lymphocytes, macrophages, polymorphonuclear cells (PMNs), foreign body giant cells and activated macrophages (Figs 4, 5). Active macrophages were defined as macrophages which had phagocytosed china ink particles. Mesothelial cells were not differentiated from macrophages as they account for only about 3% of a peritoneal cell population [10].

A student t-test was applied to assess statistical significance ($p < 0.05$). Diagrams show the average cell counts and the standard errors of the mean (Figs 6–10).

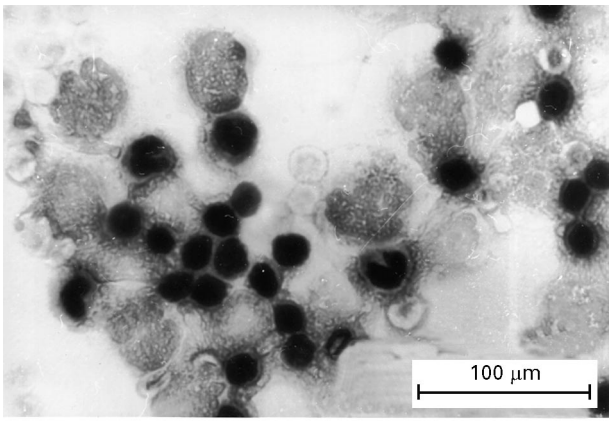

Figure 4 Light micrograph of mononuclear cells from peritoneal lavage fluid. Group B (UHMW-PE, day 14).

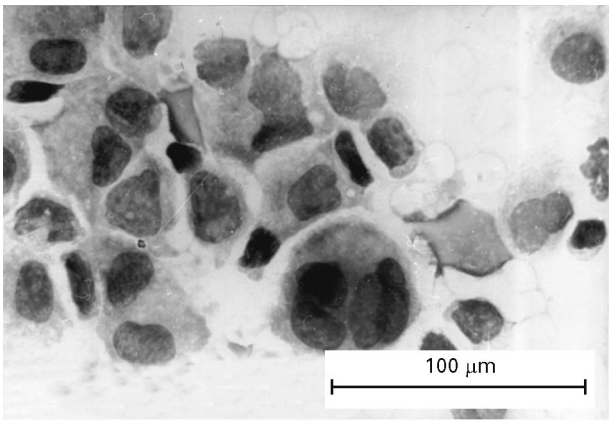

Figure 5 Light micrograph of great mononuclear cells and one giant cell with two nuclei. Group A (PET, day 7).

3. Results

The surgically treated animals showed no behavioural differences as regards moving activity compared with those untreated. No weight loss was found and none of the animals suffered from infection.

At autopsy only group B animals showed granulation tissue from the 7th day onwards. UHMW-PE powder was clumped together and was always found after sacrificing the animals whereas PET particles could only be seen in some mesenterial smears of group A mice. In some animals of group B, adherence of the gut to the abdominal wall caused intestine perforation while opening the peritoneal cavity. This was the reason why in these mesenterial smears rod-shaped bacteria were found without other signs of infection.

3.1. Number of macrophages in peritoneal lavage fluid

In group D animals (untreated controls) the average total macrophage count was about 4.4×10^6 cells (Fig. 6). All treated mice subjected to a surgical procedure showed a loss of macrophages on day 1 after treatment. This was most prominent in group B animals (0.63×10^6 cells, UHMW-PE). Animals treated with PET (group A) had 1.8×10^6 cells, and animals with a sham operation had 2.5×10^6 cells. Whereas

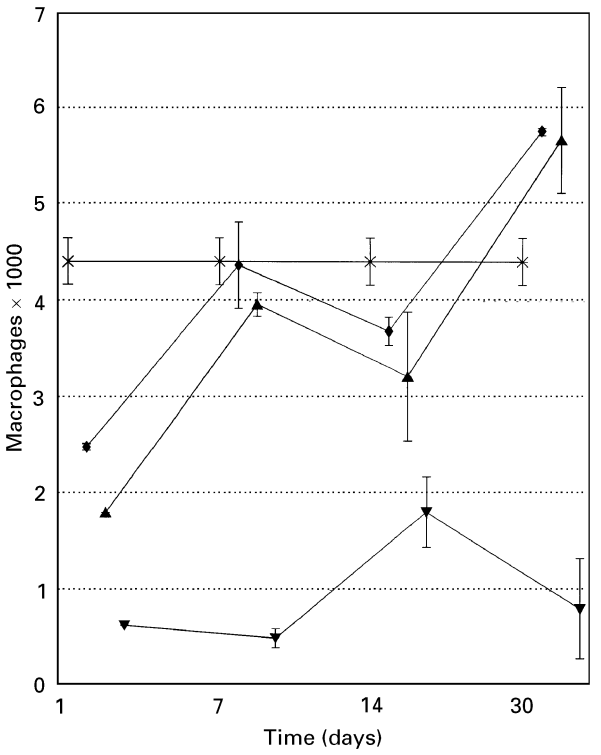

Figure 6 Number of macrophages in peritoneal lavage fluid (number +/− standard error of the mean). The loss of macrophages on day 1 recovers up to day 7 in groups A and C, whereas group B does not recover. ▲, group A (PET); ▼, group B (UHMW-PE); ◆, group C (sham operation); x, group D (untreated control).

group B mice (UHMW-PE) did not recover during the course of the study, group A and C (sham operation) reached normal cell count levels at day 7. In these two groups macrophage cell count declined again on day 14, but on day 30 higher numbers were found than in the untreated control (group D).

3.2. Small mononuclear cells: lymphocytes

On day 1 all mice subjected to a surgical procedure showed a decrease from 0.3×10^6 cells (group D: untreated control) to roughly 0.25×10^6 cells. Group B showed the smallest difference from D, while group A (PET) and C (sham operation) showed a rise in lymphocytes, with a maximum on day 14. The increase of lymphocytes in group C is the only one which is statistically significant (Fig. 7).

3.3. Polymorphonuclear cells (PMN)

On day 1 the mice treated with polymer powders showed an increase in PMN cell count from 1.1×10^6 cells in untreated animals to 1.8×10^6 (group A, PET) and 2.2×10^6 (group B, UHMW-PE) (Fig. 8). In contrast, the cell count in group C animals declined to 0.5×10^6 cells on day 1. On days 7 to 30 these mice showed a slight but significant increase in PMN cells compared to group D mice (untreated control). The PMNs of group A animals were again in the normal range at day 7 but showed a significant rise on day 30. The PMNs in the UHMW-PE mice (group B) were at 0.13×10^6 cells on day 7 and did not recover until the end of the study.

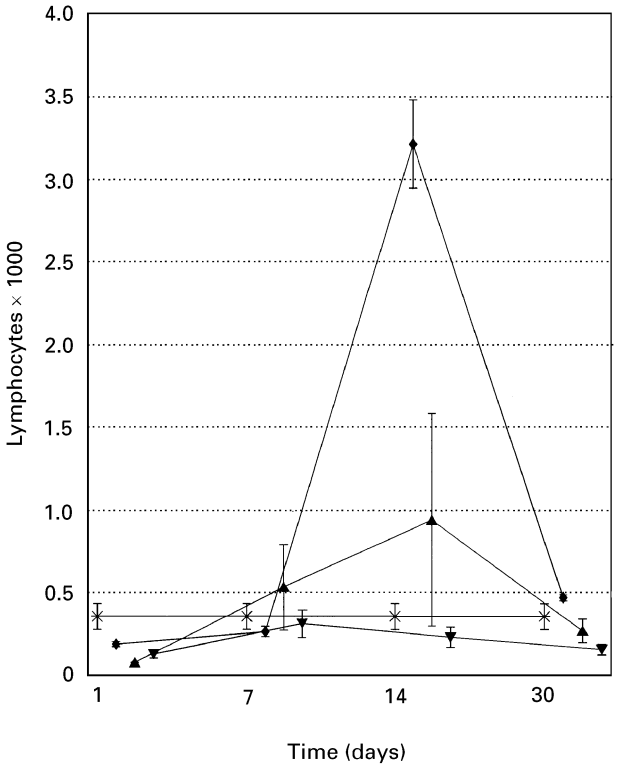

Figure 7 Lymphocyte cell count (number +/− standard error of the mean). ▲, group A (PET); ▼, group B (UHMW-PE); ◆, group C (sham operation); x, group D (untreated control).

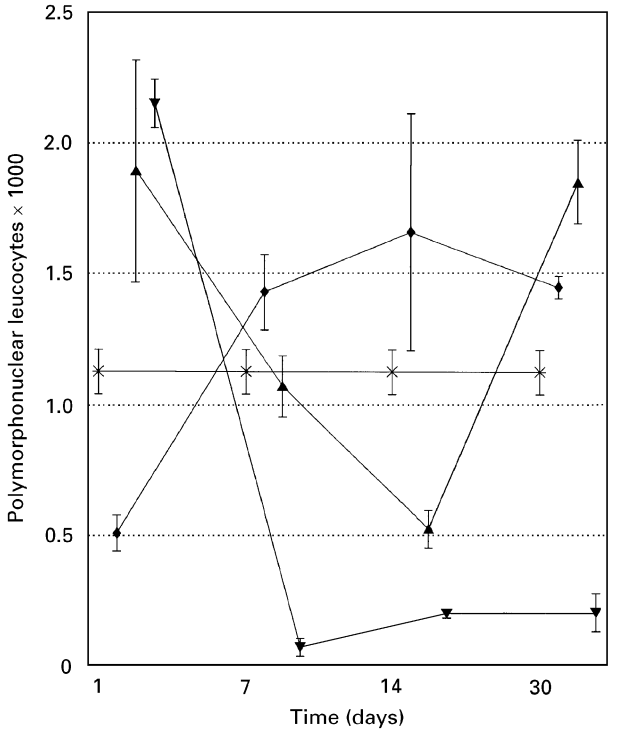

Figure 8 Polymorphonuclear cell count (number +/− standard error of the mean). ▲, group A (PET); ▼, group B (UHMW-PE); ◆, group C (sham operation); x, group D (untreated control).

3.4. Phagocytosing (active) macrophages

After 1 day the active macrophages were suppressed in all treated groups (A, B and C) (Fig. 9). Then the number of phagocytosing macrophages increased,

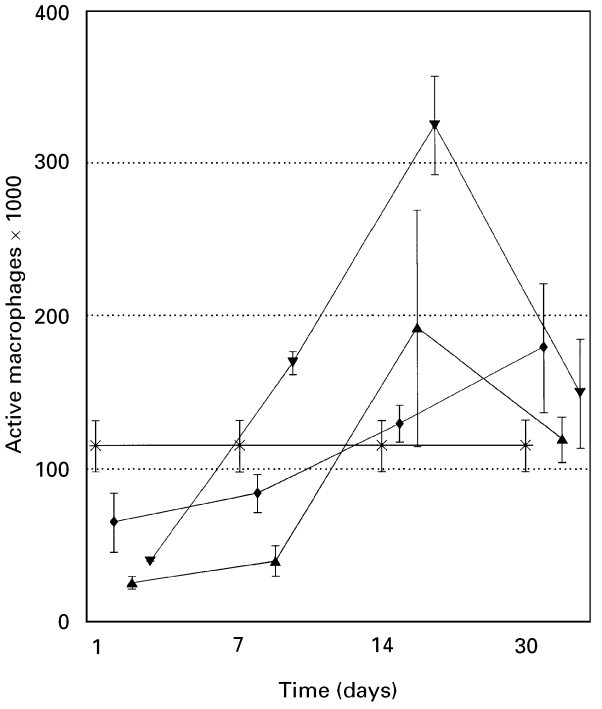

Figure 9 Number of active macrophages (number +/− standard error of the mean), counted after an incubation period of one hour. ▲, group A (PET); ▼, group B (UHMW-PE); ◆, group C (sham operation); x, group D (untreated control).

reaching a maximum on day 14 in group A and B animals, the highest being in group B (UHMW-PE). These counts returned to normal values on day 30. In mice subjected to sham operation the cell count reached their maximum on day 30.

3.5. Foreign body giant cells (FBGCs)

The first FBGCs appeared on day 7 in groups A and B. Most FBC were seen in the PET group, with a maximum cell count on day 14. But there is no statistical significance when comparing cell counts either in a group or between the different groups. In group C mice some FBCs were found on day 30 (Fig. 10). The large standard deviations are not shown.

4. Discussion

In the present study the cytological analysis of peritoneal lavage fluid was performed to assess the inflammatory response elicited by two polymer powders. These results were compared with those of a normal wound healing reaction following a midline abdominal incision, and untreated control animals. The composition and amount of peritoneal cells can be influenced by immunological or inflammatory stimuli [10–12]. Even different methods of sacrificing the animals result in different cell counts (Maurin, personal communication). Surgical procedures led to a loss of macrophages in the peritoneal fluid obtained by peritoneal lavage on day 1. This reaction was modified by application of the different polymer powders. The UHMW-PE (group B) caused a steeper slope than PET (group A) in which cell count was

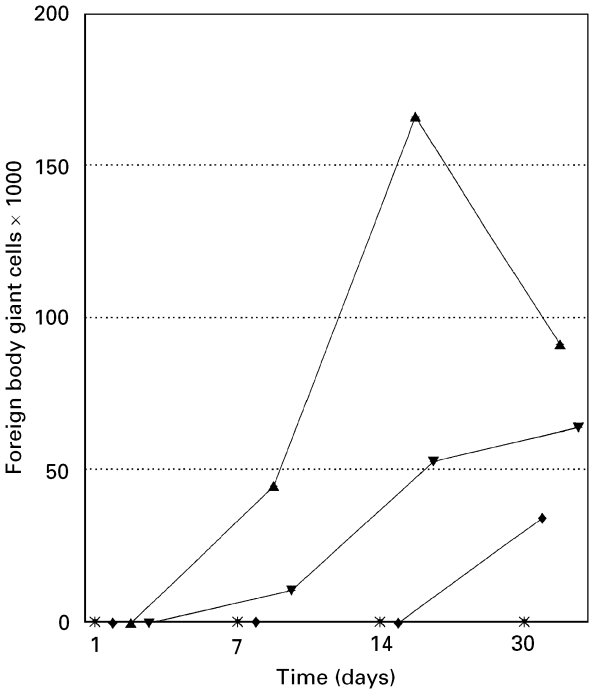

Figure 10 Number of foreign body giant cells. ▲, group A (PET); ▼, group B (UHMW-PE); ◆, group C (sham operation); x, group D (untreated control).

lower than in the sham operated mice (group C). Only mice of group B (UHMW-PE) stayed at these low cell count levels throughout the course of the study. This macrophage disappearance reaction (MDR) was described by Nelson in 1963 [11]. He demonstrated viable cells sticking to the peritoneal surface during a macrophage disappearance reaction [12]. Other authors referred to this phenomenon as leucocyte disappearance reaction (LDR) [10, 14]. While Nelson established a correlation between the rechallenge with antigen after prior sensitization and the LDR in the peritoneal lavage fluid, Bachelet described LDR as a sign of acute inflammatory response elicited by calcium-pyrophosphate injection in either the pleural or peritoneal cavity [10–12]. The rise of PMNs in the polymer-treated groups on day 1 would be in agreement with Bachelet. But on day 14 an increase in lymphocyte count was again accompanied by a loss of macrophages. This implies an immunological phenomenon as described by Nelson [11, 12]. Jonjic *et al.* found adhesion molecule expression (ICAM-1, VCAM-1) following inflammatory cytokine stimulation of mesothelial cells [15]. They showed that the number of leucocytes adhering to the mesothelial cells increased with cell adhesion molecule expression. This seems to be an important mechanism by which the macrophage/leucocyte disappearance reaction is controlled [16]. The different expression of the leucocyte disappearance reaction may be caused by the amount of adhesion molecules formed by the mesothelial cells. In the case of UHMW-PE we speculate that due to a stronger inflammatory stimulus more adhesion molecules are synthesized with the consequence of more leucocytes adhering to the peritoneal lining. Thus less inflammatory cells are obtained by the peritoneal lavage. On the other hand this may explain the granuloma formation with UHMW-PE. Our results concerning the leucocyte disappearance reaction are in accordance with those of van Sliedregt *et al.* they also describe a loss of peritoneal cells after the first study day [9]. This reaction was different for the various polymers tested: one polylactide type caused a LDR which did not resolve over the course of six months. In contrast to the polylactide particles of the van Sliedregt study, we could not find clustering after PET application. This is an interesting result and in our opinion may be a sign of a weaker inflammatory stimulus than UHMW-PE. This is in accordance with the poor biocompatibility of UHMW-PE wear products, for example in total hip endoprosthesis (aseptical loosening) [5].

Concerning the PMN count, the phase of acute inflammation was finished on day 7 in groups A and B, whereas in group C animals a slight but significant increase of PMNs was observed from days 7 to 30 with a maximum cell count on day 14. This may be attributed to the biodegradation of catgut, which usually occurs after 8 to 12 days. The moderate rise in PMN correlates with the good clinical experience gained with this suture material in patients. In contrast, the application of polymer powders seems to interfere with the response to catgut, because in groups A and B PMNs decline in this time. Not only the lymphocyte count but also the PMN count may be affected by the inflammatory response elicited by the polymers tested. Here again the binding of lymphocytes and PMNs to the mesothelium may account for this effect. And notice the resolution of LDR on day 30 and the accompanying rise of PMN in the PET group (not in the UHMW-PE group still showing LDR). This may be contributed to catgut resolution (sham operated animals also show an increase of PMNs when compared to the control group) or to degradation of PET. There are reports of PET degradation, none of these describing fragmentation in a one-month observation time [17, 18]. *In vitro* analysis revealed enzymatic disintegration of PET after esterase and papain incubation [19]. The water absorption of PET is higher than UHMW-PE. Thus hydrolytical breakdown may be facilitated. We cannot prove this thesis as we could not regain PET particles in the lavage or on macroscopical examination of the peritoneal cavity.

The chronic phase of inflammation started at day 7 (group B, UHMW-PE) and day 14 (group A, group C). An increase in active macrophage count was accompanied by a lymphocyte rise and foreign body giant cell (FBGC) formation. These results are in agreement with the findings of Marchant *et al.* that FBGC formation is correlated to the activity of macrophages [20].

UHMW-PE animals totally differed from the other groups not only in that the LDR did not resolve during the study but also that the highest number of active macrophages was found in this group. Furthermore, only in these animals was granulation tissue found at the operation site. Most probably

granulation tissue formation was a result of the high number of active macrophages and the cytokines they release to stimulate fibroblasts and adhesion molecule expression [15, 16, 21].

The reason for this strong inflammatory response of UHMW-PE cannot be fully resolved in this study. According to the particle size distribution and morphological evaluation by light microscopy the shape and size of particles are nearly the same as in PET powders. Thus geometrical factors can be excluded. Perhaps differences in surface characteristics may play an important role. But in view of the same material processing procedures for both materials the likely resulting differences in surface characteristics are polymer specific. It is reasonable that even under *in vivo* conditions such differences will be seen following the same stressing procedure. Therefore it may be misleading comparing "wear particles" of identical surface characteristics. That is why we did not perform further surface analysis. Furthermore it would be more reasonable to assume that the fibres in the PET preparation could elicit a more powerful foreign body reaction, such as observed for asbestos fibres. But instead, the course of leucocyte cell counts of mice was nearly the same as in sham operated animals. From this it can be concluded that PET in powdered form is a more physiologic biomaterial than UHMW-PE.

More research is required to elucidate the complex interactions between inflammatory cells during foreign body reaction.

With four observation times over one month the described animal model allows one to assess the acute and chronic phase of foreign body reaction [20]. Two polymer powders were characterized in terms of leucocyte disappearance reaction (LDR), PMNs, active macrophages and foreign body giant cells. Thus this model is appropriate for screening the biocompatibility of biomedical material wear products.

References

1. J. COHEN, *J. Bone Joint Surg.* **41A** (1959) 152.
2. D. L. COLEMAN, R. N. KING and J. D. ANDRADE, *J. Biomed. Mater. Res.* **8** (1974) 199.
3. S. C. WOODWARD and T. N. SALTHOUSE, in "Handbook of biomaterials evaluation", edited by A. F. von Recum (Macmillan, New York, 1986) p. 364.
4. R. D. TETIK, J. O. GALANTE and W. ROSTOKER, *J. Biomed. Mater. Res.* **8** (1974) 231.
5. S. SANTAVIRTA, Y. T. KONTTINEN, V. BERGROTH, A. ESKOLA, K. TALLROTH and T. S. LINDHOLM, *J. Bone Joint Surg. Ann.* **72** (1990) 252.
6. H. G. WILLERT, H. BERTRAM and G. H. BUCHHORN, *Clin. Orthopaed.* **258** (1990) 95.
7. J. CHARNLEY, *Clin. Orthop. Rel. Res.* **72** (1970) 7.
8. R. S. WORTMAN, K. MERRIT and S. A. BROWN, *Biomater. Med. Dev. Artif. Org.* **11** (1983) 103.
9. A. van SLIEDREGT, M. KNOOK, S. C. HESSELING, H. K. KOERTEN, K. de GROOT and C. A. van BLITTERSWIJK, *Biomaterials* **13** (1992) 819.
10. C. M. BACHELET, J. F. BERNAUDIN, J. FLEURY-FEITH and J. P. GIRAUD, *Exp. Lung Res.* **13** (1987) 241.
11. D. S. NELSON and S. V. BOYDEN, *Immunology* **6** (1963) 264.
12. D. S. NELSON and R. J. NORTH, *Lab. Invest.* **14** (1965) 89.
13. L. RENDELL-BAKER and R. B. ROBERTS, *Anesth. Analg.* **49** (1970) 919.
14. A. M. SULTAN, C. J. DUNN, P. C. MIMMS, J. P. GIRAUD and D. A. WILLOUGHBY, *J. Pathol.* **126** (1978) 221.
15. N. JONJIC, G. PERI, S. BERNASCONI, F. L. SCIACCA, F. COLOTTA, P. PELICCI, L. LANFRANCONE and A. MANTOVANI, *J. Exp. Med.* **176** (1992) 1165.
16. S. A. CANNISTRA, C. OTTENSMEIER, J. TIDY and B. deFRANZO, *Exp. Hematol.* **22** (1994) 996.
17. L. MATTURRI and E. TARDITO, *Minerva Cardioangiol.* **38** (1990) 279.
18. E. TARDITO, B. BIONDO, V. CAPUTO, G. FREDDI, E. GROSSO, S. MANTERO, A. MOTTA, M. L. REPETTI, M. C. TANZI and L. MATTURI, *ibid.* **41** (1993) 59.
19. R. SMITH, C. OLIVER and D. F. WILLIAMS, *J. Biomed. Mater. Res.* **21** (1987) 991.
20. R. E. MARCHANT, K. M. MILLER and J. M. ANDERSON, *ibid.* **18** (1984) 1169.
21. K. M. MILLER and J. M. ANDERSON, *ibid.* **23** (1989) 911.

Received 8 August
and accepted 8 August 1996