Monocyte–biomaterial interaction inducing phenotypic dynamics of monocytes: a possible role of monocyte subsets in biocompatibility

R. S. BHARDWAJ*, U. HENZE, B. KLEIN, G. ZWADLO-KLARWASSER[‡], U. KLINGE[§], CH. MITTERMAYER[¶], B. KLOSTERHALFEN[¶]
IZKF-BIOMAT, [‡]Institute of Pharmacology and Toxicology, [§]Clinic of Surgery, and [¶]Institute of Pathology, RWTH Aachen, Pauwelsstrasse 30, 52057 Aachen, Germany

For the *in vitro* study of cell–biomaterial surface interactions, the choice of cell type is crucial. *In vivo* data indicate that during the healing of the implant in the tissues, the pivotal cell types are the macrophages. These cells, upon interaction with any foreign material, might initiate a spectrum of responses, which could lead to acute and chronic inflammatory changes affecting the biocompatibility of the implant. Whether the mechanisms governing the type of evolving inflammatory reaction could be attributed to the macrophages functional differentiation mirrored by monocyte subsets during the polymer interaction, is poorly described. This *in vitro* study, therefore, attempted to investigate whether different biomaterials influence monocyte cellular activity, determined by the myeloperoxidase level and mitochondrial XTT cleavage, and phenotype dynamics characterized by the presence of CD14, RM 3/1 and 27E10 antigens. It is shown that different polymers exert differential potential to influence monocytes, both in their cellular activity and their phenotypic pattern. Thus, these findings demonstrating material-induced monocyte activation and monocyte phenotype modulation, are suggestive of the monocyte role as reporter cells in evaluating the biocompatibility of a synthetic medical device.

1. Introduction

Implantation of synthetic polymers is of increasing importance in modern medical care. The longevity and the effective functioning of the implant, i.e. biocompatibility, is determined by the events occurring at the tissue–material interface and by the surrounding environment. The biological response of the host is an acute and/or chronic inflammatory type of reaction, which is characterized by the concomitant formation of giant cells and a fibrous capsule around the implant [1, 2]. Accordingly, the magnitude, the type and the duration of the inflammatory immune response account for the biocompatibility of the material, as well as for the tissue repair and regeneration. Among the inflammatory cells, monocyte-derived macrophages have been shown to be the predominant cells at the tissue–material interface [3]. An arsenal of inflammatory mediators, cytokines and growth factors generated by these cells regulate the inflammation and wound-healing processes [4, 5].

The synthetic medical devices, with their physicochemical and mechanical properties, may influence the amplitude of the monocytes/macrophages interactions occurring at tissue–polymer interface [6]. Further, previous studies have documented that different polymers tend to elicit different inflammatory responses, such as the accumulation of monocytes/macrophages and generation of inflammatory mediators [7]. However, the mechanisms by which the implants influence the host immune response are largely unknown. Moreover, it is still unclear what molecular and cellular responses are crucial in host–material interaction and what material surface characteristics are important in mediating these responses.

In other *in vivo* studies, it was shown that the effect of net surface charge influences the polymer biocompatibility and its potential to activate monocytes/macrophages [8]. Further, polymers synthesized with non-adhesive, non-activating and bio-inert surfaces are reported to be able to suppress monocyte activation and adhesion [9, 10].

While these studies successfully demonstrate the influence of polymer biocompatibility by modifying the polymer characteristics, it also seems to be important to characterize better the monocytes/macrophages playing a central role in the inflammation process affecting material integration and longevity in

* Author to whom all correspondence should be addressed.
Selected paper from the 13th European conference on Biomaterials, Göteborg, Sweden.

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the human body. Previous reports have illustrated that different inflammatory conditions could exhibit the appearance or disappearance of distinctive monocyte subsets. For example, 27E10 (a heterodimer of two calcium-binding proteins MRP14 and MRP8) type of macrophage is prevalent in acute, yet RM3/1 macrophage is particularly observed in placenta, acute and chronic, suppressive inflammatory conditions, [11, 12]. CD14, a myeloid differentiation antigen, is described to have a critical role in binding bacterial lipopolysaccharide (LPS) to monocytes/macrophages [13]. Accordingly, the present *in vitro* study attempted to analyse the potential role of different commercially available polymers to affect monocyte phenotypes, which are analysed using confocal-laser-scan-microscopy coupled with flow cytometry to examine the modulation of these antigens on adherent and non-adherent monocyte population after contact with different polymers.

2. Materials and methods

Human buffy coats were obtained from the Blood Bank, University of Aachen, Germany. Ficoll and Percoll density gradient and other chemicals were purchased from Sigma, München, Germany, and cell culture products from Gibco BRL, Eggenstein, Germany. All of the antibodies used were received from Dianova, Hamburg. Cell culture plasticwares were obtained from Falcon, Becton-Dickinson, Heidelberg, Germany, and the hydrophobic biomembrane, Teflon membrane, was purchased from Heraeus Instruments GmbH, Osterode am Harz. The synthetic polymers used were a gift from Rehau AG. and Co, Rehau.

2.1. Monocyte purification and culture

Monocytes were prepared from human buffy coats using Ficoll and subsequent Percoll density-gradient centrifugation as described elsewhere [11]. The cells were suspended in RPMl-1640 culture medium containing 20 mM HEPES, 5% foetal calf serum and antibiotics. 2×10^6 cells/well/ml medium were cultured on LPS-free synthetic polymers in 24 well culture plates. The applied polymers were polyurethane (Raumedic-PUR 4741), polyvinylchloride 1 and 2 (PVC1 and PVC2) (Raumedic-Med 9006 and 9036, respectively) with different plasticizers (PVC1 with diethylhexylphthalate and PVC2 with triethylhexyltrimellitate), PVC1 containing tin (PVC3) (Rau-PVC7500), silicone (SIL, synthesized on the basis of methyl–vinyl–polysiloxan, Raumedic-SIK 8363), polypropylene (Raumedic-PP, 146), hydrophobic biomembrane (Teflon membrane), and polystyrene.

2.2. Confocal-laser-scan-microscopic and flow cytometric analysis

After 24, 48 and 72 h culture, non-adherent cells were separated from the adherent cells. The adherent cells were fixed with 70% ethanol. Thereafter, the cells were stained immunocytochemically for CD14, RM3/1 and 27E10 and, subsequently, examined by confocal-laser-scan-microscopy (CLSM, Zeiss, Köln, Germany), the non-adherent cells were analysed by flow cytometry (FACScan, Becton-Dickinson) as described [11]. IgG1 and IgG2 were used as isotype controls in these studies.

2.3. Spectrophotometric analysis

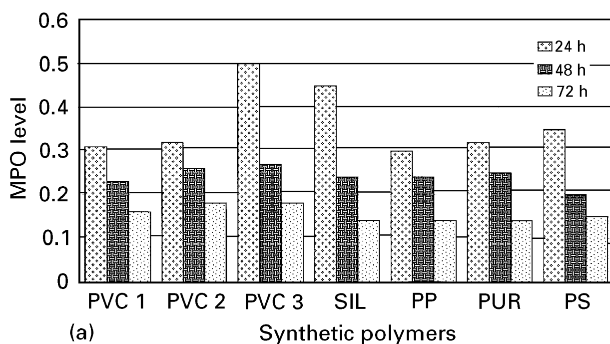
The activity of adhering and non-adhering monocytes after contact with the polymers was examined by using a myeloperoxidase (MPO) spectrophotometric method as described elsewhere [14]. Additionally, the mitochondrial activity in adherent cells was determined by the tetrazolium (XTT) spectrophotometric method [15] in which the cells were incubated with XTT for 2 h before measurement. The extinction of both of the assays was measured at 450 nm by an ELISA reader.

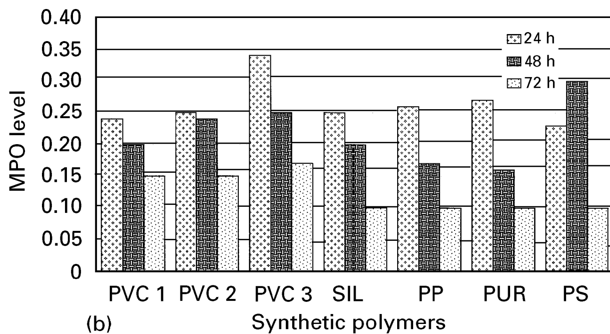
3. Results

3.1. Monocyte activity after contact with polymers

To investigate whether different polymers influence the activity of monocytes, MPO was measured spectrophotometrically in adherent and non-adherent cells cultured for 24, 48 and 72 h. Polystyrene of culture grade served as a positive control for non-toxicity and PVC3 for toxicity. As shown in Fig. 1a, 24 h culture non-adherent cells showed an increased level of MPO activity, but the magnitude differed in different polymers: PVC3 > silicone > polystyrene > PVC2 > PVC1 > polypropylene. Also, the analysis of the counterpart adherent cells showed a differing augmented level of MPO activity measured in the 24 h culture cells on different polymers, but the sequel of the magnitude observed was PVC3 > polyurethane > polypropylene > silicone > PVC2 > PVC1 > polystyrene (Fig. 1b). After 48 and 72 h culture, both adherent and non-adherent monocytes inclined to decrease the level of this activity, as observed in all the materials used, except those 48 h culture cells on polystyrene which exhibited a high increase in MPO activity, and thereafter showed a visible decline at 72 h. Moreover, the intensity of the MPO activity detected by the adherent cells was, to some extent, lower than that compared to the non-adherent cells (Fig. 1a and b). Thus, these findings could demonstrate that the degree of MPO activity of the adherent and non-adherent monocytes may be different with regard to both the different polymers in contact and the duration of culture.

In a parallel series of experiments, mitochondrial activity of the adherent monocytes was spectrophotometrically estimated by tetrazolium (XTT) metabolism. As shown in Fig. 1c, hardly any activity to metabolize XTT was detectable by only PVC3 adhering cells. Although with 24 h culture cells on PVC1, PVC2, an increased XTT cleavage activity was observed, it was found to be highly decreased by 48 h and 72 h culture cells on these polymers (Fig. 1c). In contrast, silicone, polypropylene, polystyrene and polyurethane displayed a high level of this activity,





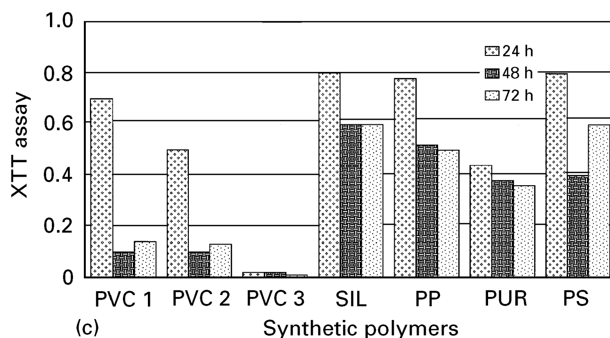


Figure 1 Monocyte cellular activity measured by MPO and XTT assay spectrophotometrically at 450 nm. Each result is the average of four experiments, in which monocytes were cultured for 24, 48 and 72 h on different synthetic polymers. (a, b) MPO level in non-adherent and adherent monocytes, respectively. (c) XTT assay of mitochondrial activity of adherent monocytes.

showing a decline after 48 and 72 h, except for polystyrene where an increase was observed at 72 h (Fig. 1c). Thus, these results illustrate that adherent monocytes may exhibit a differential potential for mitochondrial activity, depending on both the polymer type and duration of contact.

3.2. Polymer influence on monocyte phenotypes

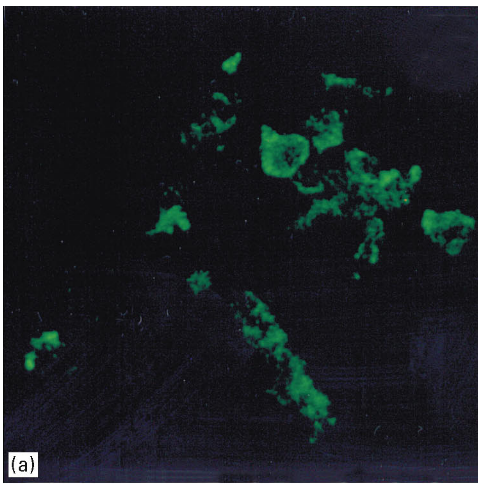
To determine whether monocytes, after contact with synthetic polymers, undergo a change in their phenotypes, monocytes were grown for 24, 48 and 72 h on different polymers. The adhering cells were analysed by confocal laser-scan-microscopy. The data obtained are presented in Table I and Fig. 2. The cells cultured on polypropylene compared to other polymers were stained highly positive only for 27E10 antigen (Table I, Fig. 2a and b), which is known to indicate the acute inflammatory type of monocyte/macrophage [11]. On the other hand, the cells cultured on PVC3 were visible as highly positive for the RM3/1 antigen (Fig. 2c, Table I), which is shown to be present on a suppressive inflammatory type of monocyte/macrophage [12]. A strong positive staining for CD14 antigen was observed only in cells cultured on polyurethane (Table I, Fig. 2e). Monocytes cultured on silicone and polystyrene showed a moderate positive staining for 27E10, RM3/1 and CD14 (Table I). This and the aforementioned pattern of staining, was visible for these three molecules with 24, 48 and 72 h culture cells. Additionally, RM3/1 as well as CD14 positive monocytes cultured by silicone showed a high propensity to fuse, thus forming a giant cell-like appearance (Fig. 2f, g), which could further be seen in CD14 positive cells on polyurethane (Fig. 2e).

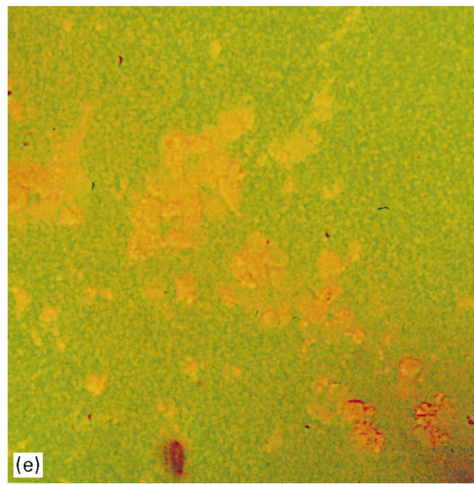
TABLE I Polymer influence on monocytic phenotypes analysed by confocal-laser-scan microscopy

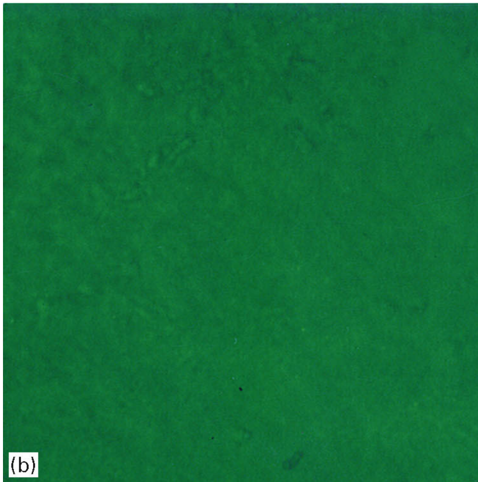
Polymer	Antibodies applied		
	27E10	RM3/1	CD14
Polypropylene	++	−	−
Polyvinylchloride[a]	+	++	(+)
Polyurethane	+	(+)	++
Silicone	+	+	+
Polystyrene	+	+	+

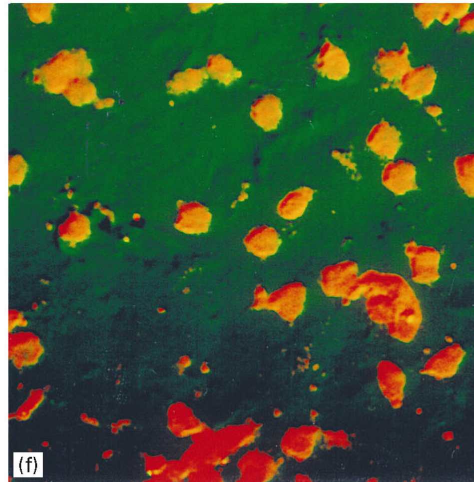
++, Highly positive; +, positive; −, negative; (+), slightly positive.
[a]PVC3, PVC1 and PVC2 showed a similar reaction to that of polystyrene.

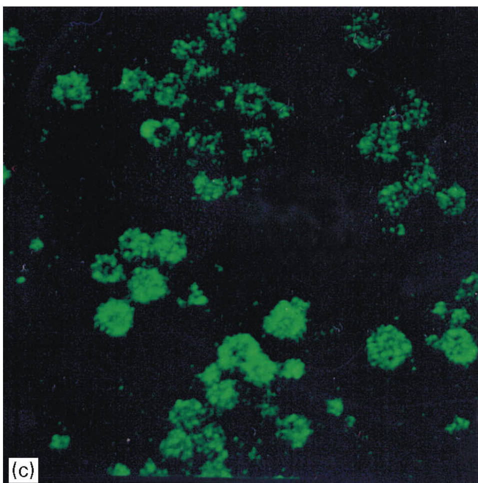
Additionally, the FACScan analysis of the 24 h non-adherent cells showed that 27E10 cell expression was increased greatly by polypropylene only, which was comparable to that of LPS-treated cells. This extent of increase was also observed by PVC3 culture cells at 48 and 72 h (Fig. 3a). In all other polymers, this augmentation was of low extent, yet prominent compared to polystyrene, showing a maximum after 72 h (Fig. 3a). The cell surface expression of RM3/1 was observed in all the monocyte cell cultures, with a notably varying level, which after 72 h by PVC3 was highly increased (Fig. 3b). In the case of CD14, monocytes cultured for 24 h on PVC1 and PVC3 exhibited a very low level of cell surface expression of this antigen, which was found to be markedly decreased by cells cultured on polypropylene and polyurethane (Fig. 3c). However, after 48 and 72 h, an increase in CD14 cell surface expression was recorded by all the polymers used, yet this increase remained absent after 72 h by PVC1. In comparison, LPS as a positive control showed an induction in the cell-surface expression of CD14 as well as in that of 27E10 (Fig. 3a and c). Thus, these results further demonstrate that the magnitude, level and time course of 27E10, RM3/1 and CD14 molecule expression on monocytes might differ when these cells are grown as adherent and non-adherent populations on different polymers.

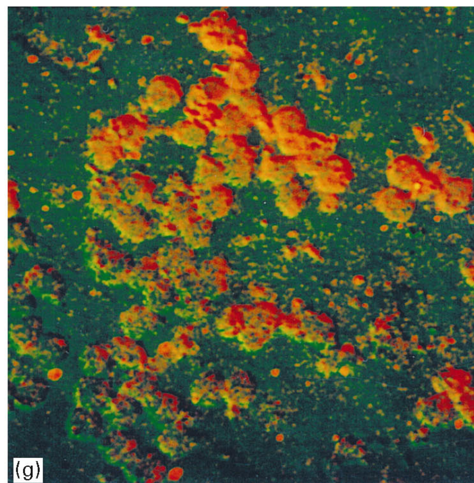












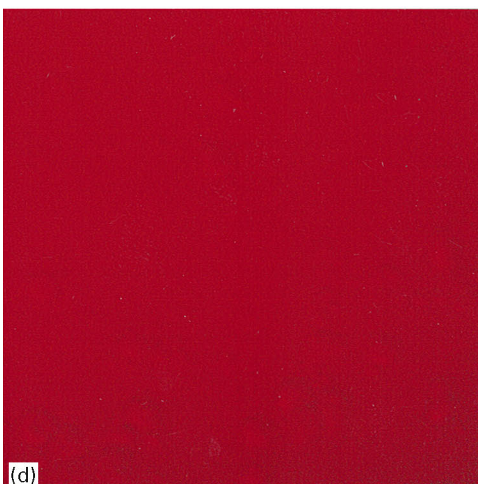


Figure 2 Confocal laser scan microscopic analysis of adherent monocytes after culture on different synthetic polymers. Monocyte phenotypes identified by the monoclonal antibodies 27E10, RM3/1 and CD14 are shown. Each result is the average of three experiments. Fusion of monocytes forming giant cell-like forms is visible in (e–g). (a) Monocytes cultured on PP stained positive for 27E10 antigen (×1000). (b) Monocytes cultured on PP stained negative for RM3/1 and/or CD14 (×1000). (c) PVC3 culture monocytes showing RM3/1 positive staining (×1000). (d) A representative isotype control (×1000). (e) PUR culture monocytes showing positive staining for CD14 (×600). (f) RM3/1 positive monocytes after culture on silicone (×1000). (g) CD14 positive monocytes after culture on silicone (×1000).

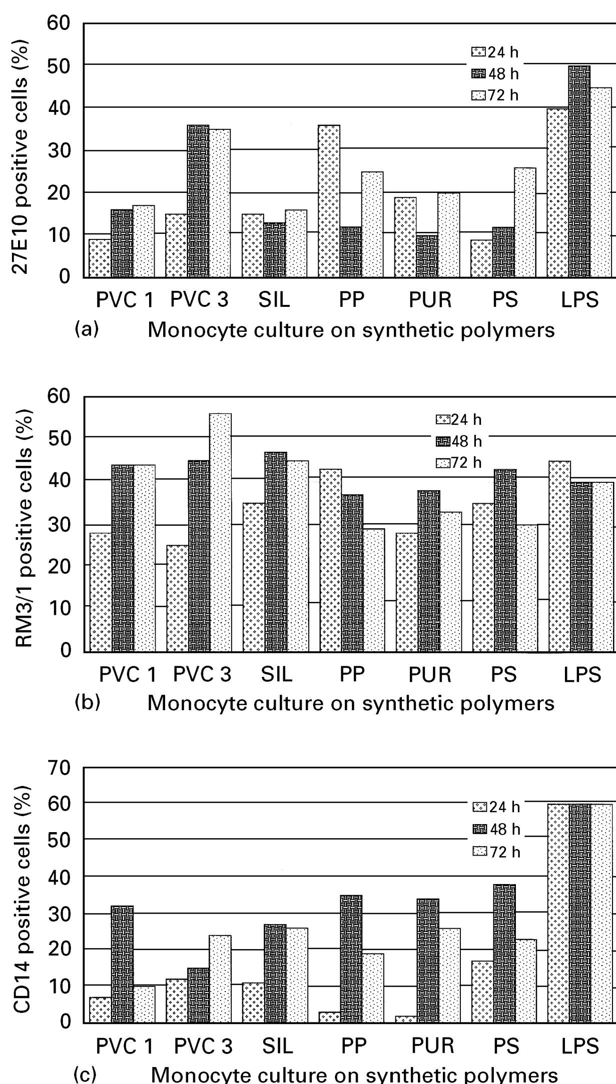


Figure 3 Flow cytometric analysis of non-adherent monocytes after 24, 48 and 72 h culture on different synthetic polymers. Each result is the average of five experiments. (a–c) Data are given as percentage of 27 E10, RM3/1 and CD14 positive monocytes, respectively.

4. Discussion

The present *in vitro* study was initiated to analyse the biomaterial-guided effects on purified human monocytes after culture on different synthetic polymers, which are commercially available and have defined clinical applications. It is shown that different materials exhibit different potential to influence both the monocyte cellular activity and phenotypes.

The monocyte cellular activity, as shown by the level of MPO, was found to be differentially affected by different polymers in a time-dependent manner. With regard to the previous studies reporting the MPO intensity as a measure for the degree of monocyte adherence on human endothelial cells [16], the difference in the MPO intensity shown in this study might correspond to the degree of monocyte adherence to the different materials, and thus lead to the opinion that different polymers could exert differential effects on the adherent capacity of monocytes. This is further supported by the observation that for the 48 h culture, adherent monocytes on a culture-grade non-toxic plastic (polystyrene), demonstrated an increased level of MPO compared to other materials. Accordingly, an augmented number of adhering monocytes is suggested, which could not be ruled out by this study. Additionally, monocyte populations with different adherence potential, e.g. early and late adhering monocyte subsets, may exist, which supports the view that this adherence property of different monocyte subsets could only be visible with the high-grade bio-inert polystyrene. Considering the fact that all the polymers used in this study are defined to be bio-inert, except PVC3, this effect, as also described in another study [17], might then be the result of a non-physiological stimulus by different plastics, indicating that the polymer type may influence differentially the monocyte adherence.

Moreover, MPO is shown to be an integral enzyme of the myelomonocyte lysosomal apparatus. The observation that the monocyte MPO varied after adherence is indicative of the degree of activation related to the duration of contact time with the material. Consistent with the idea that the adherence process activates the monocytes [18–20] and the increasing exposure time may decrease the monocyte MPO responsiveness, it is more likely that material-induced activation may result in a progressive depletion of lysosomal granules and thus MPO. Accordingly, monocytes after contact with non-toxic materials may be secured, while with toxic material the monocytes may be exhausted, during the activation. Similar observations have also been reported by previous *in vivo* studies evaluating variations in the activity of cellular acid phosphatase and aminopeptidase in cryostat sections of different implant sites [21].

Additionally, the observed lack in monocyte mitochondrial activity, but fairly high MPO activity, after contact with the toxic PVC3, corresponds to the idea that monocytes encountering the biomaterial may not be appreciably altered. This opinion further finds support in another study [22] and in the similar results presented here after various durations of the different monocyte cultures with the non-toxic PVC1 and PVC2. Also, the observation that 72 h culture monocytes on polystyrene exhibit a high mitochondrial activity, emphasizes the idea that there is no alteration in monocyte activity. However it could also be possible that monocyte responses have various transduction pathways that also depend on the type and duration of stimulus. These and the results discussed above, thus suggest that synthetic polymers exert differential effects on the heterogeneous characteristics of monocytes and indicate that, while defining the biocompatibility of a polymer, the choice of cell type and methods to investigate the cellular activity, is crucial. This seemingly bewildering array of different monocyte functions necessitates careful evaluation of the experimental design and the monocyte subpopulation under investigation.

Peripheral blood monocytes constitute a very heterogeneous cell population. A large amount of effort has been made to characterize the various monocyte phenotypes by applying different isolation techniques [17, 23, 24] and recently by monoclonal antibodies [11, 12, 25]. In different acute and chronic

inflammatory conditions, a predominant subset of monocytes has been recognized by the monoclonal antibodies 27E10 and RM3/1 (11, 12). Recently, these and CD14 antigen have been described to play a role in monocyte adhesion and migration [11, 16, 26]. Different previous *in vivo* studies demonstrated the varied appearance of monocyte/macrophages at the tissue–material interface [27, 28]. Also a recent report [29] on abdominal-wall replacement by mesh implantation show that the degree of inflammation and fibrosis depends on the nature of the material-induced tissue reaction, where monocytes/macrophages are pivotal interacting cells. According to these observations, polypropylene inducing a significant acute inflammatory reaction, also preferentially induces 27E10 antigen-bearing monocytes, as detected by both confocal laser scan microscopic and flow-cytometric analysis in the present study. Further, the recent finding that toxic PVC3 potentially increases the RM3/1 and decreases 27E10 and CD14 antigen in monocytes agrees with the idea that highly necrotic, toxic inflammatory conditions may require the presence of inflammation down-regulatory type of macrophages, preventing further tissue damage in the body. Additionally, the observation of monocyte fusion to form the giant cell-like appearance shown by RM3/1 and CD14 positive monocytes adherent on silicone and polyurethane, as well as the preferential increased level of CD14 positivity on polyurethane adherent monocytes, may also corroborate with the idea material-induced activation and organization of monocytes during foreign-body reaction [1, 3].

Studies [30, 31] proposing that adherence provides inductive signals to monocytes and renders them capable of responding to other signals support thus the notion that monocytes may represent reporter cells for such material-primed activated monocytes/macrophages. Additional signals, depending on the tissue environment, involvement of adhesion structures and soluble mediators for the differentiation of monocytes, will be crucial for the biocompatibility of a material in medical care.

5. Conclusion

These results demonstrate that different synthetic polymers have different potentials differentially to affect monocyte cellular activity and phenotypes. Monocyte phenotypes may be "reporter" cells for the type of biomaterial in use and appear to be important in evaluating the biocompatibility of the implant. The molecular rationale responsible for this effect is under investigation.

Acknowledgement

The authors thank Mrs S. Korr and Mr L. Muys for their valuable assistance with tissue culture, CLSM and flow-cytometry. This work was supported by a BMBF grant project no. 01KS9503/9 for IZKF-BIOMAT RWTH Aachen.

References

1. Q. H. ZHAO, J. M. ANDERSON, A. HILTNER, G. A. LODOEN and C. R. PAYET, *J. Biomed. Mater. Res.* **26** (1992) 1019.
2. W. J. KAO, Q. H. ZHAO, A. HILTNER and J. M. ANDERSON, *ibid.* **38** (1994) 73.
3. T. N. SALTHOUSE, *ibid.* **18** (1984) 395.
4. J. M. ANDERSON, *Cardiovasc. Pathol.* **2** (1993) 3:35S.
5. C. SUNDERKÖTTER, K. STEINBRINK, M. GOEBELER, R. BHARDWAJ and C. SORG, *J. Leukoc. Biol.* **55** (1995) 410.
6. J. M. ANDERSON, *ASAIO* **11** (1988) 101.
7. S. A. M. ALI, P. J. DOHERTY and D. F. WILLIAMS, *Biomaterials* **15** (1994) 779.
8. J. A. HUNT, B. F. FLANAGAN, P. J. McLAUGHLIN, I. STRICKLAND and D. F. WILLIAMS, *J. Biomed. Mater. Res.* **31** (1996) 139.
9. K. M. DEFIFE, J. K. YUN, A. AZEEZ, S. STACK, K. ISHIHARA, N. NAKABAYASHI, E. COLTON and J. M. ANDERSON, *ibid.* **29** (1995) 431.
10. R. KAPUR, J. LILIEN, G. L. PICCIOLO and J. BLACK, *Ibid.* **32** (1996) 133.
11. R. S. BHARDWAJ, C. ZOTZ, G. ZWADLO-KLARWASSER, J. ROTH, M. GOEBELER, K. MAHNKE, M. FALK, G. MEINARDUS-HAGER and C. SORG, *Eur. J. Immunol.* **22** (1992) 1891.
12. G. ZWADLO, R. SCHLEGEL and C. SORG, *J. Immunol.* **137** (1986) 512.
13. S. D. WRIGHT, R. A. RAMOS, P. S. TOBIAS, R. J. ULEWITCH and J. C. MATHISON, *Science* **249** (1990) 1431.
14. E. M. E. VERDEGAAL, I. BLOCKLAND, H. BEEKHUIZEN and R. VAN FURTH (eds.), in "Mononuclear Phagocytes" (Kluwer Academic, Dordrecht, The Netherlands, 1992). p. 123.
15. T. MOSMANN, *J. Immunol. Method.* **65** (1983) 55.
16. I. WENZEL, J. ROTH and C. SORG, *Eur. J. Immunol.* **26** (1996) 2758.
17. C. A. OWEN, E. J. CAMPBELL and R. A. STOCKLEY, *J. Leukoc. Biol.* **51** (1992) 400.
18. L. NORDSLETTEN, A. K. M. HOGASEN, Y. T. KONTTINEN, S. SANTAVIRTA, P. ASPENBERG and A. O. AASEN, *Biomaterials* **17** (1996) 1521.
19. G. C. ZENNI, *J. Invest. Surg.* **7** (1994) 135.
20. K. MAHNKE, R. BHARDWAJ and C. SORG, *J. Leukoc. Biol.* **57** (1995) 63.
21. R. E. MERCHANT, K. M. MILLER and J. M. ANDERSON, *J. Biomed. Mater. Res.* **18** (1984) 1169.
22. S. S. KAPLAN, R. E. BASFORD, E. MORA, M. H. JEONG and R. L. SIMMONS, *ibid.* **26** (1992) 1039.
23. A. BOYUM, *Scand. J. Immunol.* **17** (1983) 429.
24. C. A. OWEN, M. A. CAMPBELL, S. S. BOUKEDES, R. A. STOCKLEY and E. J. CAMPBELL, *Amer. J. Physiol.* **267** (1994) 267.
25. K. ODINK, N. CERLETTI, J. BRÜGGEN, R. G. CLERC, L. TARCSAY, G. ZWADLO, G. GERHARDS, R. SCHLEGEL and C. SORG, *Nature* **330** (1987) 80.
26. H. BEEKHUIZEN, I. BLOKLAND and R. VON FURTH, *J. Immunol.* **150** (1993) 950.
27. C. GRETZER, A. S. ERIKSSON, B. ALLDEN, L. E. ERIKSON and P. THOMSEN, *Biomaterials* **17** (1996) 851.
28. A. S. ERIKSSON, L. E. ERIKSON and P. THOMSEN, *J. Mater. Sci. Mater. Med.* **5** (1994) 269.
29. B. KLOSTERHALFEN, U. KLINGE, U. HENZE, R. BHARDWAJ, J. CONZE and V. SCHUMPELICK, *Langenbecks Arch. Chir.* **382** (1997) 87.
30. D. F. EIERMAN, C. E. JOHNSON and S. HASKILL, *J. Immunol.* **142** (1989) 1970.
31. A. D. YUROCHKO, D. Y. LIU, D. EIERMAN and S. HASKILL, *Proc. Nat. Acad. Sci. USA* **89** (1992) 9034.

Received 5 May
and accepted 12 May 1997