Role of HSP70i in regulation of biomaterial-induced activation of human monocytes-derived macrophages in culture

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The functioning of an implant depends on the material properties and the wound-healing process. The latter is led by an inflammatory reaction guided mainly by monocyte/macrophage activity. This *in vitro* study investigated human monocytes/macrophages in culture from 2 h to 10 days on silicone, polyurethane, teflon and TCPS. Analysis of cytokine release by ELISA showed that maturing macrophages have different capacities to produce cytokines TNFα, IL10, IL8 and GM-CSF. The long culture-mature macrophages on all polymers produced comparable low levels of TNFα, IL10 and IL8. Monocytes/macrophages on polyurethane and teflon, and those on silicone only in long culture-time produced high GM-CSF amounts, where as those on TCPS exhibited low levels of GM-CSF. FACS analysis revealed that HSP70i was highly inducible after short time culture yet this high level was maintained in long culture-mature macrophages on TCPS only, whereas on other polymers the mature macrophages showed a high reduction in HSP70i level, which demonstrated a high stress-response by cells on TCPS. Accordingly, CLSM-analysis revealed low nuclear NF-κB in cells on TCPS and high nuclear NF-κB in mature macrophages on silicone and polyurethane, showing a high cellular activation on the latter two polymers. This corresponded also to the high mitochondrial activity by XTT metabolism displayed by the mature macrophages on polyurethane $\geq$ silicone $>$ teflon $>$ TCPS. These data show a correlation of (1) cytokines (TNFα, GM-CSF) and HSP70i, (2) NF-κB and HSP70i by monocytes/macrophages after contact with polymers. Thus, HSP70i might be a useful molecular candidate for exploring biomaterial-induced inflammatory reaction.

1. Introduction

Besides the documented advantages of the recently available medical devices, there are several reports on the prevailing complications after implantation. This resides in the fact that biomaterials as foreign bodies in the human organism induce an inflammatory reaction, namely a foreign body type of reaction, which appears not only to be responsible for the integration of the implant, but also its susceptibility to infection [1]. A major component of this inflammatory system consists of monocytes/macrophages. These cells are capable of migration, adherence, aggregation, chemotaxis and phagocytosis [2, 3]. These processes are enhanced in response to external inflammatory stimuli and accordingly, these cells are activated to produce a series of inflammatory mediators [4–6]. The ongoing inflammatory process and secretory repertoire of the infiltrating monocytes apparently need to be highly regulated, i.e. from the healing process to the induction of fibrosis and granuloma formation [7, 8]. The regulation may be closely associated with the migration, adhesion, activation and differentiation of the monocytes to macrophages in a given environment [9, 10].

The key players in these processes are described to be the cytokines [11, 12]. In particular, the IL8 induces chemotaxis and release of granule enzymes [13, 14], up-regulation of adhesion and formation of bioactive lipids [15, 16]; GM-CSF plays a crucial role in survival, proliferation, cytocidal activities and differentiation of the monocytic/phagocytic lineage [17–19]; TNFα is an immediate-early multifunctional cytokine initially identified as mediating necrosis of solid tumors of mice [20, 21] and lysis of transformed cells *in vitro* [22, 23] and thus, confer the macrophage with cytotoxic capabilities; IL10 execute profound anti-inflammatory functions through macrophage deactivation and inhibi-

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tion of the production of inflammatory cytokines like TNFα and IL8 [24–27] and thus bestow macrophages with suppressive abilities.

The monocyte/macrophage responses are expected to occur when blood contacts synthetic materials used in hemodialysis, cardiopulmonary bypass, heart valves, artificial abdominal wall reconstruction, artificial joints and vascular grafts. Several studies have reported that different polymers tend to induce monocytes/macrophages to secrete cytokines [28], proteolytic enzymes [29] and reactive oxygen mediators [30]. The level of activation of these cells to produce these inflammatory mediators might be influenced by the different physicochemical and mechanical properties of the polymers [31–33]. Some studies have suggested that certain host proteins or protein fragment(s) adsorbed to the biomaterial surface may affect the accumulation and activation of inflammatory cells [34]. Other *in vitro* and *in vivo* studies indicate the role of various functional subsets of monocytes/macrophages activated by the cell-material interactions [35, 36].

Despite detailed knowledge of the specific properties exhibited by various monocyte/macrophage subsets, little is known about the mechanisms contributing to this specialization in the different monocyte/macrophage subpopulations. A recent study reported that NF-κB-like transcription factors may play a role in different macrophage subpopulations [37]. Also a large body of evidence implicates NF-κB-like transcription factors in the macrophage activation, such as induction of gene encoding cytokines, reactive oxygen species and adhesion molecules [38–40].

The observations that NF-κB is induced during cellular activation under inflammatory conditions has led to the search for NF-κB suppressing mechanisms under anti-inflammatory conditions. Here a major role has been ascribed to the anti-inflammatory cytokines IL10, IL4 and IL13 [41, 42]. Further, inhibitory effects on cellular activation have also been shown by the stress response proteins, especially the heat shock proteins (HSPs) of the HSP70 family, consisting of constitutive HSP70 (HSP70c) and inducible HSP70 (HSP70i) [43–45]. A recent study in lung epithelium states that heat shock response inhibited inducible nitric oxide synthase (iNOS) gene expression in a NF-κB deactivation mechanism [46, 47]. Additionally, induction of HSP70i synthesis as a cellular protective response is correlated with macrophage activation under diverse stimuli. Heat-treated macrophages with highly induced HSP70i synthesis show a restricted release of TNFα and IL1 after LPS stimulus [48], M-CSF induction of HSP70i synthesis in macrophages renders them resistant to oxidative stress [49]. Further, the search for an intracellular player has shown that endogenous TNFα is responsible for the induction of HSP70i synthesis [50]. Whether or not these molecular mechanisms are essential in the activation of monocytes/macrophages with respect to polymers or the other factors is unknown.

The objective of the present study was to study monocyte differentiation in culture with different polymers regarding the production of HSP70i and cytokines, and the activation of NF-κB and the oxidative metabolism. We could find that these activities are differently regulated during the monocytes transformation to macrophages *in vitro*.

2. Materials and methods

Human buffy coats were obtained from the Blood Bank, University of Aachen, Germany. Ficoll and Percoll were purchased from Sigma, Muenchen, Germany and cell culture products from Gibco BRL, Eggenstein, Germany. All of the enzyme-linked immunoabsorbent assay (ELISA) reagents used were received from Biozol, Muenich, Germany. Fluorescein-isothiocynate (FITC)-conjugated (green fluorescence) monoclonal antibodies (mAbs) defining CD14 and CD3, isotype controls and detection antibodies (FITC- and Phycoerytherin (PE)-conjugated (red fluorescence)) were purchased from Coulter-Immunotech, Hamburg, Germany. The mAb directed against HSP70i was obtained from Biomol, Hamburg, Germany and affinipurify polyclonal antibody (Ab) against NF-κB from Santa Cruz Biotechnology, California, USA. Cell culture plastic wares were obtained from Falcon, Becton-Dickinson, Heidelberg, Germany and the hydrophobic biomembrane (Teflon membrane) was purchased from Heraeus Instruments GmbH, Osterode, Germany. The synthetic polymers used were a kind gift from Rehau AG and Co., Rehau, Germany.

The applied polymers were silicone (Raumedic-SIK 8363 and ployurethane (Raumedic-PUR 4741). The tissue culture grade biomembranes, Teflon and TCPS were employed as hydrophobic and hydrophilic non-toxic controls respectively.

2.1. Monocytes purification as adherent cells and culture

Monocytes were prepared from fresh human buffy coats using Ficoll and subsequent Percoll density gradient centrifugation as described elsewhere [51]. The monocytes obtained were suspended in RPMI-1640 culture medium containing 20 mM HEPES, 10% heat-inactivated pooled human serum and antibiotics. These cells were further purified by plastic adherence. One million cells/ml medium were allowed to adhere on culture grade plastic plates for 30 min at 37 °C, 5% CO_2. Thereafter, the non-adherent cells were decanted followed by 3 × washing with PBS (37 °C). This cell fraction was subjected once more to plastic adherence on fresh dishes. The adherent monocytes were treated with cold PBS containing 10% fetal calf serum (FCS) and 4 mM Ethylene-Diamine-Tetra-Acetic acid (EDTA) for 15 min on 0–4 °C and thereafter carefully scraped off from the surface of the culture plates by the use of a rubber policeman. The viability and purity of the cells was tested by dye exclusion test and FACScan-Analysis, respectively. All cell preparations constituted of 97–99% CD14 positive monocytes, whereas no CD3 positive cells were detectable.

One million cells/ml medium/well were cultured on LPS free synthetic polymers in six well culture plates for 2 h, 2, 4, 6, 8, and 10 days. One ml medium was added to the d 3 and d 7 cultures. At every culture period 200 µl supernatant was collected to quantify the cytokines secretion of TNF-α, IL-8, IL-10 and GM-CSF.

2.2. Measurement of contact angle

Contact angle measurement for silicone, polyurethane, teflon and TCPS was performed according to the captive-bubble-method by applying goniometermicroscope G-23 (Krüss, Hamburg, Germany). The experiments were repeated three times. For each polymer in an experiment the measurements were repeated ten times.

2.3. FACS-analysis

The FACS-Analysis (Calibur, Becton-Dickinson, Heidelberg, Germany) of the purified adherent monocytes was performed as described elsewhere [51]. Antibodies against CD14 (Isotype IgG2b) and CD3 (Isotype IgG1a) were applied as specific monocytes and T cells marker, respectively. In parallel appropriate isotype controls were used in these experiments. For the FACS-analysis of HSP70i, the monocytic cells after culture (see Section 2.1) and washing (2 × with PBS, 37 °C), were harvested as described in Section 2.1, transferred to a 50 ml Falcon tube, and treated with an equal volume of 4% formaldehyde (20 min, room temperature (RT)). Thereafter, the cells were washed (2 × with PBS, 37 °C) and permeabilized with PBS buffered 0.5% TritonX-100 (1 ml, 3 min, 4 °C) and again washed with PBS. The cells thus treated were stained for HSP70i with the specific mAb and FITC-conjugated detection antibody and analyzed by FACScan.

2.4. Confocal-laser-scan-microscope (CLSM) analysis

The monocytic cells at different culture time points (see Section 2.1.) after washing (2 × with PBS, 37 °C), were fixed (1 ml 4% formaldehyde (20 min, RT), permeabilized with PBS-buffered 0.5% TritonX-100 (1 ml, 3 min, 4 °C) and again washed with PBS. The cells thus treated were double stained for HSP70i and NF-κB with the specific Abs, which were visualized by using PE and FITC-conjugated detection antibodies respectively, by CLSM analysis.

2.5. Spectrophotometric analysis

The cytokine secretory activity of monocytes/macrophages after contact with the polymers was determined spectrophotometrically by using commercially available specific ELISAs (Biozol-Endogen, Germany) for TNF-α, GM-CSF, IL-8 and IL-10.

The mitochondrial activity of monocytes/macrophages after contact with the polymeres was examined using the Tetrazolium (XTT) spectrophotometric method in which the cells were incubated with XTT for 2 h before measurement. The extinction of both of the assays was measured as 450 nm by an ELISA reader.

2.6. Statistics

Each experiment was carried out at least six times and statistic analysis was performed using a one-way analysis of variance (ANOVA) and Wilcoxon Matched-Pairs Signed-Ranks test ($p < 0.05$).

3. Results

The present study investigated the pattern of HSP70 expression, cytokine release, nuclear mobilization of NF-κB and mitochondrial activity in human monocytes during their maturation to macrophages in *in vitro* culture on synthetic polymers, silicone, polyurethane, tissue culture grade teflon (Biomembrane) and polystyrene (TCPS). The contact angle measurements showed that these polymers exhibit different contact angle degrees corresponding to their hydrophobic character in the following order: teflon > silicone, polyurethane > TCPS (Fig. 1). Silicone and polyurethane showed a similar contact angle degree and thus similar degree of hydrophobicity. On these polymers, monocytes/macrophages were recovered about 90% of the seeded cells. The viability of the recovered cells was always higher than 90% (Dye exclusion test).

3.1. HSP70i production in culture-mature macrophages on polymers

A different pattern of HSP70i positivity was recorded during monocytes/macrophage differentiation on different polymers (Fig. 2). Compared to the isolated monocytes, the number of HSP70-positive monocytes was highly increased by the 2 h culture on polymers in the following order: teflon and TCPS > silicone > polyurethane (Fig. 2). After 2 h the number of HSP70-positive monocytes/macrophages decreased, except on polyurethane, which showed a decline not earlier than at d 4, reaching a minimum at d 8 to d 10 (Fig. 2). The decrease by teflon and silicone was prominent, showing a minimum at d 4 and was visible with minor fluctuations till d 10 (Fig. 2). In contrast, little change was observed in the number of HSP70 positive cells on TCPS till d 10 (Fig. 2).

Time course study further showed that the most suitable time for HSP70 induction for monocytes/macrophages alike, lay in the period 2 h to d 2 (Fig. 2).

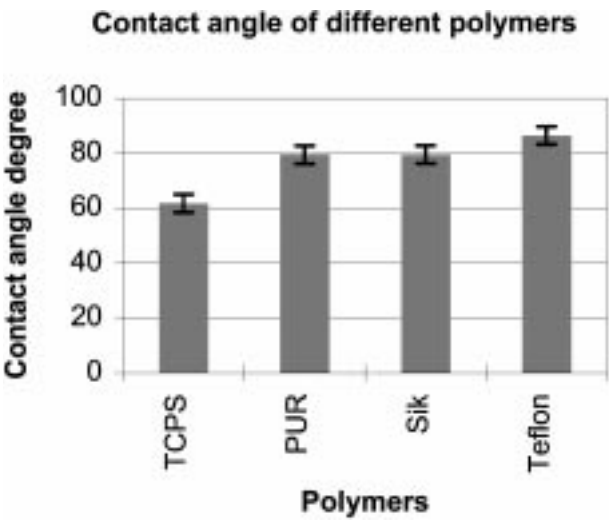


Figure 1 Contact angle measurements by adapting the captive-bubble-method using goniometermicroscope G-23 (see the Materials and Methods section). The data shown is the average ± SD of three experiments. The polymers exhibit different contact angle degrees corresponding to their degree of hydrophobicity such as teflon > silicone (SIK), polyurethane (PUR) > TCPS.

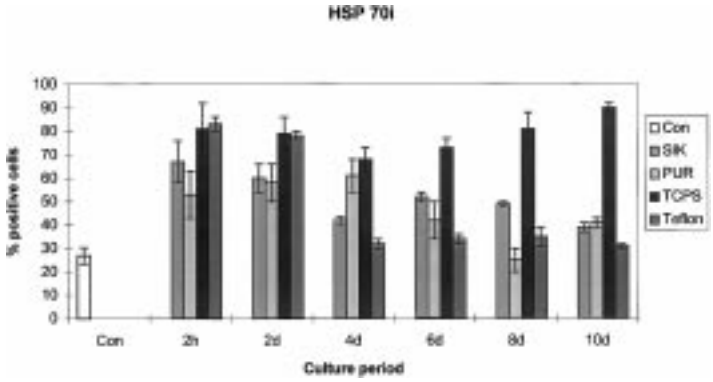


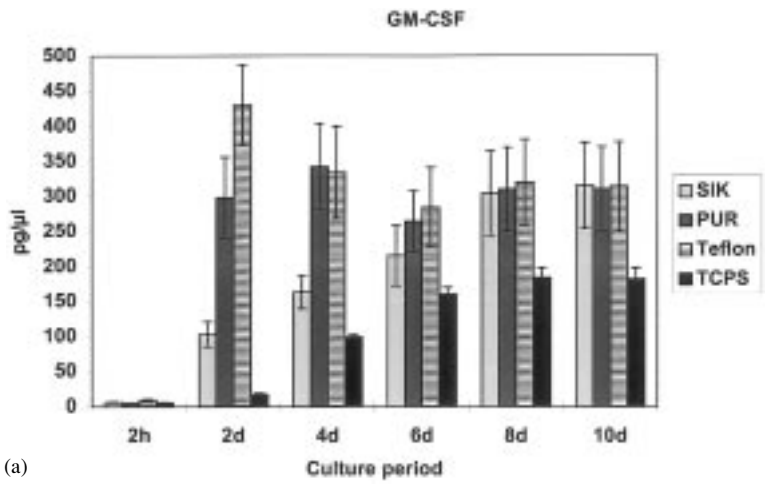
Figure 2 FACS analysis of HSP70i production in adherent monocytes-derived macrophages in 2 h–10 d in culture with silicone (SIK), polyurethane (PUR), TCPS and teflon. Monocytes immediately after density gradient purification are shown as control (Con). The cells were fixed and permeabilized as described in the Materials and Methods section. Both monocytes and macrophages on TCPS show high levels of HSP70i, whereas on other polymers macrophages display low levels of HSP70i compared to monocytes. Each result is the average ± SD of five experiments. The significance was determined by ANOVA variance analysis and Wilcoxon Matched-Paris Signed-Ranks test, $p < 0.05$.

The number of HSP70-positive monocytes/macrophages was high, yet to different degrees by different polymers at the early stage of 2 h to d 2 (Fig. 2). Compared to TCPS which revealed almost a similar number of HSP70-positive monocytes/macrophages, the number of HSP70-positive mature macrophages was reduced by other polymers, however, to a different magnitude in the following order: teflon > polyurethane > silicone (Fig. 2).

3.2. Cytokine production in culture-mature macrophages on polymers

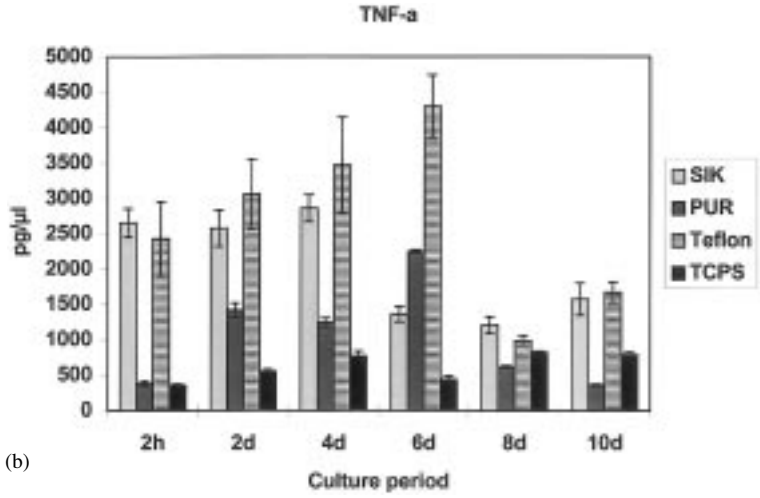
A different secretory activity pattern was recorded for each of the cytokines TNFα, IL8, IL10 and GM-CSF examined during cell maturation on different polymers. High GM-CSF amounts were detected from d 2 to d 10 by cells on polyurethane and teflon, though the latter was accompanied by a minor reduction. Conversely, the cells on silicone and TCPS showed increasing levels of GM-CSF, which were of lower magnitudes by the d 2 to d 4 culture-mature monocytes/macrophages, and higher in d 8 to d 10 culture-mature macrophages (Fig. 3a). High amounts of TNFα were detected by silicone and teflon culture cells at 2 h to d 4 and d 6, respectively, and were reduced by d 8 to d 10 culture-mature macrophages. In contrast, the polyurethane and TCPS cultures showed low levels of TNFα. However, polyurethane showed a maximum TNFα at d 6, which also declined by mature cells at d 8 to d 10 (Fig. 3b). Similarly, IL10 levels produced by differentiating monocytes on all polymers were increased from 2 h to d 4 or d 6 although with varying degrees. Further, this high IL10 level was reduced, except in cells on TCPS, to the initial 2 h IL10 level by macrophages at d 8 to d 10 on silicone and teflon and at d 10 on polyurethane, respectively (Fig. 3c). Comparably, IL8 showed its maximum at d 2, which decreased thereafter. This decrease was of the highest degree by the monocytes/macrophages culture on silicone. However, an increase in IL8 level was observed by d 10 mature cells on all polymers, showing similar magnitudes with only minor variations (d 3).

Time course studies showed that 2 d supernatants conformed best for the different cytokines released by monocytes/macrophages. The peak concentrations of cytokines were reached at different time points with different polymer cultures (Fig. 3a–d). Also the different cytokines showed timely extended peaks, although this



(a)

Figure 3 Cytokines release by adherent monocytes-derived macrophages in culture on silicone (SIK), polyurethane (PUR), TCPS and teflon. The supernatants of 2 h to 10 d culture were collected and analyzed by specific cytokines ELISAs. Each result is the average ± SD of six experiments. The significance was determined by ANOVA variance analysis and Wilcoxon Matched-Pairs Signed-Ranks test, $p < 0.05$. (a) GM-CSF, (b) TNFα, (c) IL10 and (d) IL8 release respectively.



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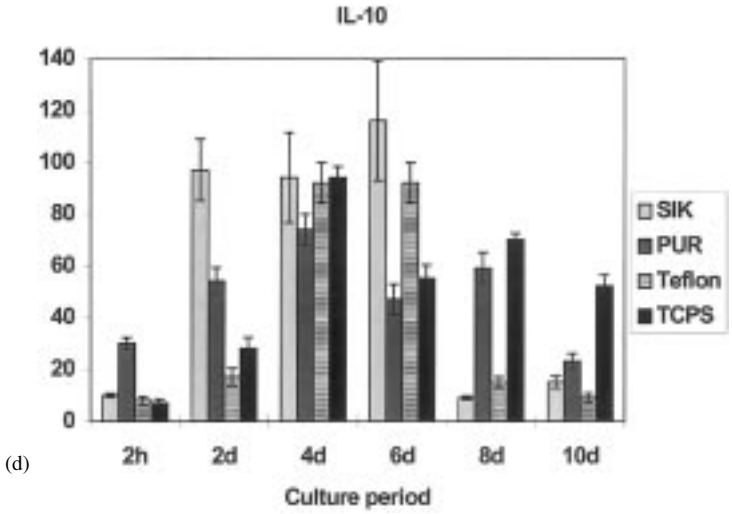

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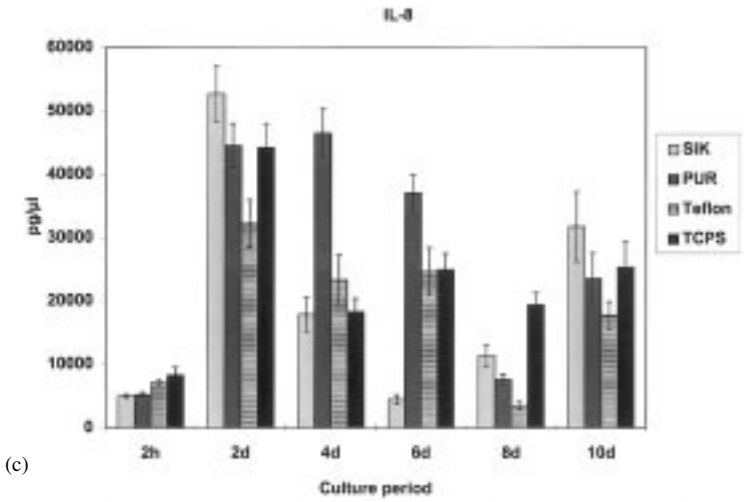

(c)

Figure 3 (Continued).

pattern varied by different polymers. Comparing the HSP70i production and cytokines release, we found that cultures with high levels of HSP70i positive cells secreted low levels of TNFα. This inverse correlation was prominent by the TCPS and polyurethane culture cells. The cultures on other polymers, silicone and teflon also showed similar results, however, only confined to certain culture time points, silicone at d 4 and teflon at d 6. In contrast, GM-CSF levels paralleled with the HSP70i positivity, however, again confined to certain time points on different polymers: teflon, polyurethane and TCPS at d 2 to d 10, silicone at d 6 to d 10, respectively. Similarly, IL8 levels also showed a direct correlation to the HSP70i positivity, however, this was

more pronounced in d 2 to d 6 culture-mature monocytes/macrophages on all the polymers. Conversely, no such correlation was observed for IL10 and HSP70i.

3.3. NF-κB translocation in culture-mature macrophages on polymers

A different pattern of NF-κB translocation was detected in monocytes maturing to macrophages on different polymers (Table I, Fig. 4a–c). Both short and long time-culture monocytes/macrophages on TCPS showed a weak staining for nuclear NF-κB (Table I, Fig. 4c). Conversely, silicone and polyurethane exhibited strong staining for nuclear NF-κB, which was even stronger in the d 6 to d 10 macrophages on silicone and polyurethane (Table I, Fig. 4a–b). Apparently, the magnitude of the nuclear NF-κB staining in cells cultured on silicone was higher compared to the other polymers (Table I). This study also showed that the high HSP70i positivity at 2 h to d 2 or d 4 corresponded to the low degree of nuclear presence of NF-κB in silicone and polyurethane culture cells (Table I, Fig. 4b). The macrophages on silicone and polyurethane at d 8 to d 10 showed less HSP70i positivity and high nuclear presence of NF-κB (Table I, Fig. 4a). By TCPS, both short and long time-culture monocytes/macrophages alike, showed a high level of HSP70 positivity with low levels of nuclear NF-κB (Table I, Fig. 4c). Further, these HSP70i results obtained by CLSM are similar to those shown by FACS analysis as shown above.

3.4. Oxidative activity of culture-mature macrophages on polymers

In other series of experiments, mitochondrial activity was spectrophotometrically analyzed by tetrazolium (XTT) metabolism. As shown in Fig. 5, enhanced XTT metabolic activity from 2 h to the maximum at d 4 and d 6 was detected in maturing monocytes grown on TCPS and teflon, respectively. This level was maintained with minor reductions till d 10. This activity increased in cultures on silicone and polyurethane, showing its maximum at d 10 (Fig. 5). Further, the time course study demonstrated that d 4 mitochondrial activity by different polymers is well suited for the cellular oxidative metabolism for monocytes/macrophages. The highest level of XTT metabolic activity was detected only by the d 10 culture-mature macrophages on silicone and polyurethane, the latter with macrophages exhibiting a still higher activity (Fig. 5). Comparing the HSP70i production and the XTT metabolic activity, we found that the high HSP70i level corresponded to the low XTT metabolic activity and vice versa, and thus demonstrating an inverse correlation of HSP70i and oxidative metabolism.

4. Discussion

The present *in vitro* study was set-up to examine the material-induced effects on monocytes differentiating to macrophages in culture on different synthetic polymers, which are commercially available and have defined clinical applications. We could demonstrate (1) that for the secretory activity, the stage of differentiation is of particular importance, (2) that each of the cytokines investigated adapted a different pattern during cell differentiation on different polymers, (3) that prolonged culture leads to the generation of macrophages that already constitutively secrete immunoregulatory cytokines, (4) that activation of NF-κB translocation and mitochondrial metabolism depends on the stage of macrophage differentiation and the substrate, and (5) that for the induction of HSP70i the process of adhesion, the stage of macrophage differentiation and the hydrophilicity-hydrophobicity of substrates are substantial.

Other studies with Hela cells have shown that the hydrophilic substrates induce HSP70 expression stronger than the hydrophobic ones [52], which agrees with our results shown by d 3 to d 10 culture mature macrophages on TCPS. However, the 2 h to d 2 monocytes/macrophages both on hydrophilic and hydrophobic substrates showed increased level of HSP70i. Further, the monocytes/macrophages in 2 h through d 10 culture were seen to be adherent as observed microscopically, though the mature macrophages showed lowering level of HSP70i. This observation, at least in mature macrophages, shows that HSP70i production does not necessarily correlate with the cellular adhesion, which is in agreement to that reported by others in Hela cells showing that HSP70 induction is independent of cellular adherence [52]. The different high HSP70i levels in the early monocytes/macrophages culture (2 h–2 d) additionally support the opinion that the adherence to surfaces with different properties, i.e. silicone and polyurethane with micro-

TABLE I CLSM analysis of HSP 70i and NF-κB-p52 in the monocytes-derived macrophages after contact with different polymers on various culture periods

Culture	Silicone HSP70i	NF-κB	Polyurethane HSP70i	NF-κB	TCPS HSP70i	NF-κB
2 h	+ +	(+)	+ +	(+)	+ + +	(+)
2 d	+ +	+ +	+ +	+	+ + +	+
4 d	+	+ +	+ +	+	+ +	+
6 d	+	+ + +	+	+ +	+ +	+
8 d	(+)	+ + +	(+)	+ +	+ + +	(+)
10 d	(+)	+ + +	(+)	+ +	+ + +	(+)

+ + + = very highly positive, ++ = highly positive, + = positive, (+) = slightly positive, h = hours, d = days.

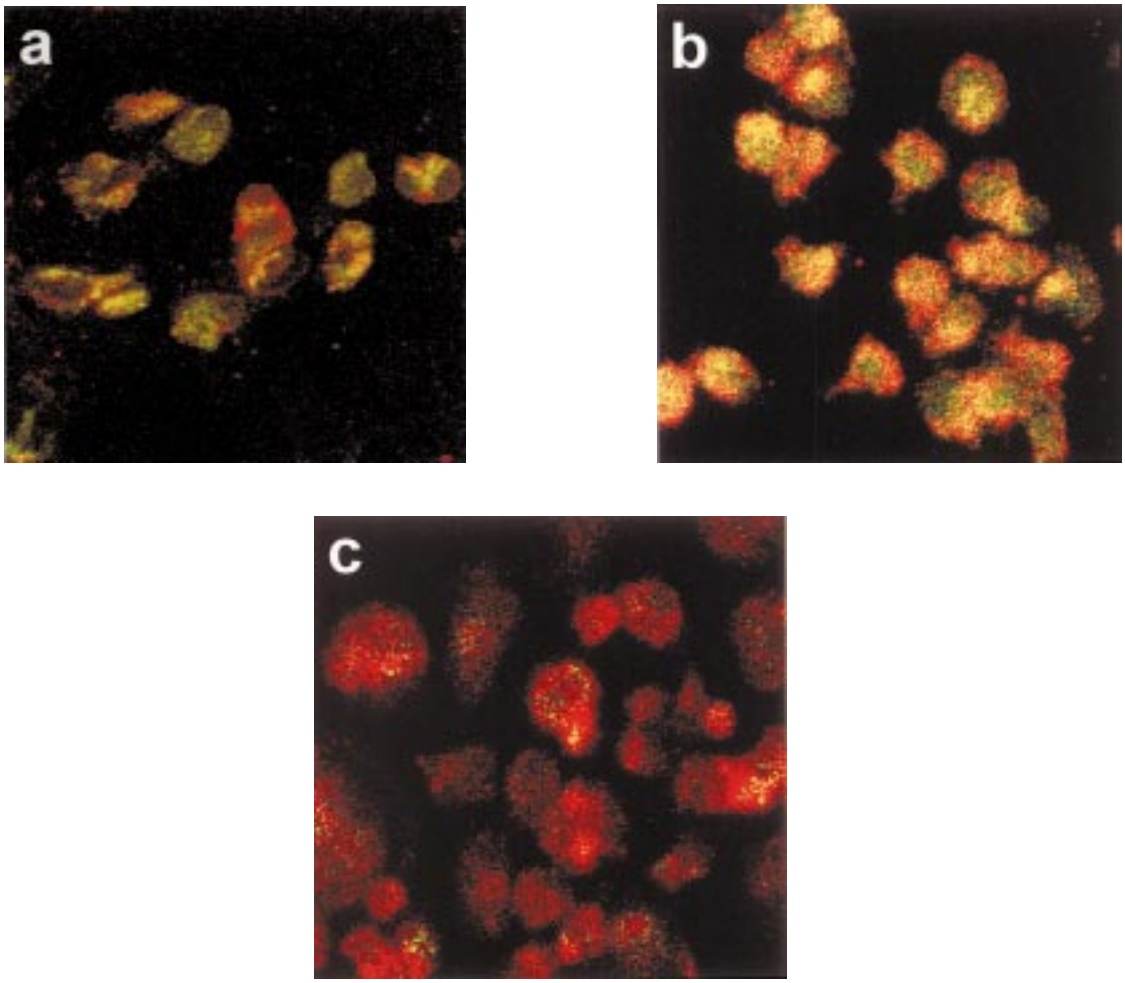

Figure 4 CLSM analysis of double-fluorescence staining of NF-κB (green) and HSP70i (red) in adherent monocytes-derived macrophages in culture on silicone, polyurethane and TCPS. (a) 8 d to 10 d macrophages (× 1650) on silicone or polyurethane with high nuclear NF-κB (green), low levels HSP70i (red) and cytoplasmic NF-κB/HSP70i (orange). (b) 2d mature monocytes (× 1850) on silicone or polyurethane with different levels of NF-κB (green), HSP70i (red) and NF-κB/HSP70i (orange). (c) monocytes and macrophages (× 1750) on TCPS with low nuclear as well as cytoplasmic NF-κB (green) and high levels of HSP70i (red).

rough surface on the one hand, and teflon and TCPS with smooth surface on the other hand, may also affect the HSP70 expression. The similar high levels of HSP70i at 2 h to 2 d in cells on the both hydrophobic teflon and hydrophilic TCPS could also be traced back to the similarities in their smooth surface properties. The results of early high (2 h–2 d) and late low (d 4–d 10) levels of HSP70i in cells on silicone, polyurethane and teflon compared to almost unaltered high levels of HSP70i in cells on TCPS show that the cells on TCPS obviously suffered from a high stress response. Whether this resulted from the divergent monocytes differentiation to macrophages on different surfaces, could not be ruled out; although, the expression of HSP70 has been associated with cell differentiation during gene activity in early mouse embryos [53] and *in vitro* differentiation

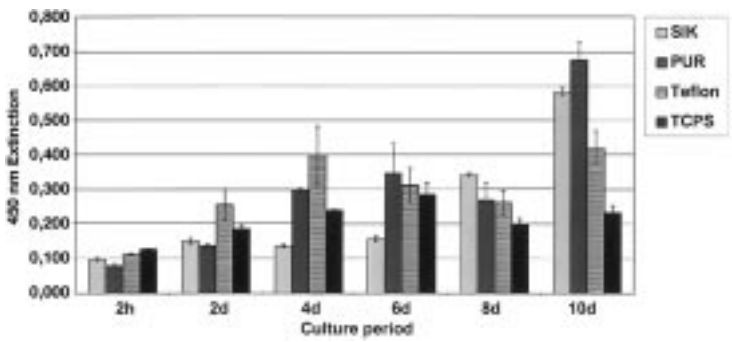

Figure 5 Monocyte-derived macrophages mitochondrial activity measured by XTT assay spectrophotometerically at 450 nm. Each result is the average ± SD of five experiments, in which the cells were cultured for 2 h to 10 d on silicone (SIK), polyurethane (PUR), TCPS and teflon. The significance was determined by ANOVA variance analysis and Wilcoxon Matched-Pairs Signed-Ranks test, $p < 0.05$.

of established haemopoetic cell lines [54, 55]. Further, It is not quite clear what kind of signal is transduced to the cells; however, though unknown, selective response with different magnitude occurred in monocytes/macrophages after they adhered to hydrophilic or hydrophobic surfaces. Presumably, the different protein adsorption properties and physicochemical characters of the surfaces might play a role. Similar observations have also been made in other studies on hydrophobic and hydrophilic surfaces effecting protein adsorption, cell adhesion, cell proliferation [56, 57] and HSP70 expression [52].

Additionally, *in vitro* studies have reported that biomaterials with different surface coatings could affect monocyte adhesion and cell activation to produce cytokines like IL1, TNFα and IL6 [32]. Another *in vivo* study has shown that surface charge can influence the early phase acute inflammatory response such as macrophage infiltration and high TNFα production at d 2 [31]. The material-induced effect is also visible in the present study, demonstrating different secretory patterns and levels of the cytokines TNFα, IL8, IL10 and GM-CSF in cells on different polymers. Further, monocytes/macrophages grown on silicone compared to polyurethane, though with similar degrees of hydrophobicity and microrough surface, secreted at 2 h to 2 d high levels of TNFα, IL8 and IL10. IL8 secretion was highly downregulated on silicone after d 2 compared to that on polyurethane. In contrast, GM-CSF levels were constantly high on teflon and polyurethane, while on silicone and TCPS the secretion of GM-CSF increased with culture time.

The secretory activity of d 8 to d 10 culture-mature macrophages showed a similar down-regulatory course for pro-inflammatory cytokine TNFα and anti-inflammatory cytokine IL10 on teflon, silicone and polyurethane. Conversely, IL10 secretion on TCPS at d 6 to d 10 sustained at low levels, although higher compared to those at 2 h to d 2. The secretion of chemokine IL8 appeared to be biphasic as it again increased in d 10 culture-mature macrophages. In general, the d 8 to d 10 culture-mature macrophages compared to 2 h to d 6 culture-mature macrophages on all polymers, showed almost a similar pattern but different levels of secretion, particularly for TNFα, IL8 and GM-CSF. These results indicate that in addition to the surface properties, there might be unknown factor(s) influencing the monocyte differentiation and activation to produce cytokines. The adherence guided signal(s) are known to influence the differentiation and activation of monocytes/macrophages [58]. Expression of a number of genes of monocyte early inflammatory mediators (IL1 β, IL8, TNFα, CSF-1, Superoxid dismutase) have been reported to be more dependent on the selectivity of signals induced by adherence to different substrates [59]. Further, the regulation of cytokine release mediated by gene expression occurs at multiple levels. For example, different post-transcriptional modifications might be involved, as shown for M-CSF receptor [60]. Also the cytokines autocrine as well as the cytokines cross-talk regulatory loops might be of importance, e.g. TNFα increases the production of IL10 and the latter inhibits the TNFα production [61].

Further, the transcription factor NF-κB described to modulate the cellular activation process of cytokine and oxidative mediator production is activated by adherence [62]. This study showed high nuclear presence of NF-κB in culture-mature macrophages on silicone and polyurethane, corresponding to the XTT oxidative metabolism activity of these cells. This indicates that macrophage mitochondrial activity is augmented on these polymers. Conversely, the monocytes/macrophages with low nuclear NF-κB on TCPS correspond to the low XTT oxidative metabolism, and thus a low degree of mitochondrial activation. Additionally, the teflon culture showed that the high mitochondrial activity observed in the maturing monocytes/macrophages was sustained in the d 8–d 10 culture-mature macrophages. Obviously, the non-phagocytoseable hydrophobic surfaces have higher oxidative potential compared to hydrophilic ones, as is also known from other studies [63, 64], though the opposite has also been reported [65]. However, these results lend further weight to the opinion that in addition to the role of surface and chemical properties and adhesion specific signal(s), unknown factors might be involved in the monocyte differentiation and activation on polymers. Nevertheless, divergent activation of monocyte subtypes [35] present in the adherent cells population on different substrates could also be assumed. Other studies also hold the view that the macrophage populations with distinctly active NF-κB-like transcription factors may also exist [37]. These views could also be supported by the different cellular activity of monocytes/macrophages observed in this study.

Moreover, the induction of NF-κB and HSP70i is involved in cytokines and oxidative mediators production [38, 40, 46, 47]. This study illustrates that production of HSP70i is associated with that of cytokines; a reciprocal relationship with TNFα and a direct correlation with GM-CSF, which corresponds to reports from other studies [48, 49]. Whereas a direct correlation for the IL8 and HSP70i production in cells on silicone and polyurethane is suspected, no such relation is visible for IL10. A relation of NF-κB and cytokines could not be shown by this study, though IL10 could be supposed to be responsible for the activation of NF-κB in d 8 to d 10 culture-mature macrophages on silicone, polyurethane and TCPS. This is in agreement with the study reporting NF-κB suppression by IL10 [41]. Further, the oxidative metabolism utilizing reactive oxygen species is suggested to be a proximal effector for the NF-κB activation [40]. HSP70i is implicated in the inhibition of the NF-κB activation and thus the genes expression responsible for the cellular oxidative activity [46, 47]. This is in line with the present findings showing that the higher the cellular HSP70i level, the lower the nuclear NF-κB and the mitochondrial redox activity and vice versa. These and the aforementioned points underline the central role of HSP70i in the immunobiology of monocytes/macrophages after contact with different surfaces. The low HSP70i production in macrophages on hydrophobic surfaces implies a suppression of this cellular stress response activity and an activation of cellular oxidative potential. Similar macrophage activity might account for the defluorization of the teflon vascular grafts as shown earlier [66]. The regulatory molecular rational for this

remains a moot point. Studies on the induction of a putative immediate-early and late differentiation gene as well as a signal transduction stage may provide more insights in the activation of monocytes/macrophages interacting with polymers.

5. Conclusion

Apparently, the HSP70i is a suitable molecular candidate to study the adherence guided differentiation of monocytes to macrophages on different surfaces. The immediate-early phase of adherent monocytes with higher HSP70i levels, both in hydrophobic and hydrophilic surfaces, suffered higher degrees of heat shock like stress-response; conversely, only the macrophages on hydrophobic surfaces with lower HSP70i levels suffered lower degrees of such response. Accordingly, this study emphasizes that the investigation of HSP70i in the versatile monocytes maturing to macrophages, and its role in the cellular actions guided by NF-κB-like transcription factors, cytokines, oxygen mediators and adherence may be useful not only in defining the biocombatibility of polymers, but also influence the survival and function of these cells in the unfavorable inflammatory conditions.

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