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Cytokine profiling using monocytes/macrophages cultured on common biomaterials with a range of surface chemistries

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Abstract

Cytokines, chemokines, and growth factors were assayed from the supernatants of monocytes and macrophages cultured on common biomaterials with a range of surface chemistries. TNF- α , MCP-1, MIP-1 α , IL-8, IL-6, IL-1 β , VEGF, IL-1ra, and IL-10 were measured from monocyte/macrophage cultures at different stages of activation and differentiation seeded onto polyethylene, poly-urethane, expanded polytetrafluoroethylene, polymethyl methacrylate, and a hydrogel copolymer of 2-hydroxyethyl methacrylate, 1-vinyl-2-pyrrolidinone, and polyethylene glycol acrylate in tissue culture polystyrene (TCPS) plates. Empty TCPS wells and organo-tin polyvinyl chloride served as “blanks” and positive controls, respectively. Results showed an overall increase in cytokine, chemokine, and growth factor production as monocytes are activated or differentiated into macrophages and that proinflammatory and anti-wound healing cytokines and chemokines dominate this profile. However, cytokine production was only modestly affected by the surface chemistry of these four stable and noncytotoxic biomaterials.

Keywords

biomaterial; cytokine; macrophage; foreign body reaction; *in vitro*

INTRODUCTION

The interaction between immune cells and the surface of a material, termed the foreign body reaction, is believed to determine, or at least strongly influence, the eventual success or failure of an implanted device.¹ The foreign body reaction is composed of two phases: (1) an inflammatory phase immediately following implantation that neutralizes pathogens and removes damaged tissue, and (2) a repair phase that includes the healing and reconstitution of the implant site through regeneration of damaged tissue at the injury site.¹ Although the inflammation and wound healing phases are characterized by a variety of cell types including neutrophils, fibroblasts, endothelial cells, and platelets, the predominant cell type at the surface of a long-term implant is the monocyte-derived macrophage.²

It is commonly held that the performance of an implanted material is dependent on the cascade of intercellular signals that originates from monocyte-derived macrophages at the

implant surface.¹ Macrophages at an implant surface secrete cytokines, chemokines, and growth factors that signal the recruitment and activation of lymphocytes, fibroblasts, endothelial cells, and smooth muscle cells and that orchestrate the formation of new tissue around implanted devices.¹ Cytokine activation of lymphocytes also leads to the fusion of macrophages into foreign body giant cells that have been shown to persist throughout the lifetime of an implanted device and are known to attack implant surfaces with degradative enzymes.² A number of these cytokines, chemokines, and growth factors have been categorized as either pro or anti in terms of their inflammatory or wound healing character in the foreign body reaction.³ The characterizations in Figure 1 have been adapted from the original source³ to include vascular endothelial growth factor (VEGF), which is considered proinflammatory and pro-wound healing for its role in promoting both dilation of blood vessels to maintain inflammation as well as angiogenesis to revascularize damaged tissue.⁴ Macrophage inflammatory protein (MIP-1 α) and monocyte chemoattractant protein-1 (MCP-1) were also included in this figure as proinflammatory and anti-wound healing chemokines for their roles in recruiting monocytes, macrophages, neutrophils, T-lymphocytes, and inducing other proinflammatory cytokines.^{5,6}

One goal of the current study was to investigate the effect of monocyte activation and differentiation on cytokine production. *In vitro* investigations of the immune response rely on monocyte/macrophage cultures, either from primary sources or established cell lines.⁷⁻⁹ For our studies, an immortalized human monocyte cell line (THP-1) was utilized to assess cell-biomaterial interactions.¹⁰ Though, the untreated THP-1 monocyte cell line has been shown to exhibit different characteristics, such as surface marker expression and cytokine production, from primary monocytes/macrophages isolated from peripheral blood, the addition of activation or differentiation factors promotes THP-1 monocytes to more closely resemble primary monocytes/macrophages.¹¹⁻¹⁴ Phorbol 12-myristate-13 acetate (PMA) is known to differentiate THP-1 cells into macrophage-like cells, which resemble native monocyte-derived macrophages with regard to morphology, membrane receptor and antigen expression, and production of secretory products.¹² In contrast, lipopolysaccharide (LPS) is generally considered an initiator of classical activation in monocytes/macrophages, rather than a differentiator.¹⁵ Classically activated monocytes/macrophages migrate to sites of inflammation where they encounter and degrade pathogens, though they are not necessarily more phagocytic than resting cells.¹⁶ PMA- and LPS-treated cells, in addition to untreated cells, were analyzed in this study for their impact on cytokine production in response to biomaterials.

Another goal of this study was to investigate the effect of surface chemistry, using commonly implanted materials, on the production of a range of cytokines known to be involved in inflammation and wound healing. Currently, there are two seemingly paradoxical views of surface mediated effects on the tissue surrounding implanted materials. One view, that is, the “Anderson Hypothesis,” purports that certain biomaterial surface chemistries may dictate patterns of cytokine production, presumably affecting the outcome of the foreign body reaction.^{3,7} Another argument, that is, the “Ratner Hypothesis,” suggests that healing surrounding an implant is essentially invariant 1 month postimplantation for many common biomaterials, regardless of surface chemistries, as long as the material is

smooth, chemically inert, and nondegradable.¹⁷ Recent studies provide evidence to support either a surface chemistry-dependent immune reaction^{3,7,18–21} or an immune reaction independent of surface chemistry.^{22,23} To date, there remains no comprehensive correlation between bio-material surface chemistry, cytokine production, and the outcome of the foreign body reaction.

The current study determined the initial cytokine profiles produced by human monocytes/macrophages (THP-1) with varying degrees of activation or differentiation seeded onto common biomaterials that cover a range of surface chemistries. Untreated, LPS-treated, and PMA-treated human monocytes/macrophages were seeded onto polyethylene (PE), polyurethane (PU), expanded polytetrafluoroethylene (ePTFE), polymethyl methacrylate (PMMA), and a hydrogel copolymer of 2-hydroxyethyl methacrylate, 1-vinyl-2-pyrrolidinone, and polyethylene glycol acrylate (HEMA-PEG). Organo-tin stabilized polyvinyl chloride (ot-PVC), which is recommended by the International Organization for Standardization as a positive control for cytotoxicity, was included as the positive control.²⁴ In addition, since all measurements were performed in 24-well tissue culture polystyrene (TCPS) plates, empty wells of TCPS were employed as the “blank.” The production of cytokines, chemokines, and growth factors, including TNF- α , MCP-1, MIP-1 α , IL-8, IL-6, IL-1 β , VEGF, IL-1 α , and IL-10, by monocytes/macrophages were examined over 48 h for correlation with the different biomaterials. Results indicate that cytokine production was significantly affected by monocyte activation and differentiation, but only modestly affected by the surface chemistry of these stable and noncytotoxic biomaterials.

METHODS

Biomaterials

High-density PE (US Pharmacopeia, Rockville, MD), PU/pellethane 2363–80AE-050824, a soft segment PU based on polyether and aromatic isocyanate (PU; Polyzen, Apex, NC), ePTFE (Bard, Tempe, AZ), PMMA (Modern Plastics, Bridgeport, CT), and ot-PVC (Smiths Medical, Hythe, UK) were cut into 1-cm² squares and sonicated for 20 min in 70% ethanol. The materials were then rinsed with pyrogen-free water, dried with lint-free wipes, and sonicated in pyrogen-free water for 20 min. These cleaned materials were then gas-sterilized with ethylene oxide and outgassed for 8 h at 135°F. Sterilized materials were tested for the presence of endotoxin using the QCL-1000 endotoxin assay. Endotoxin levels of all of the sterilized materials were less than the FDA-approved limit for medical devices (0.5 EU/mL).

HEMA-PEG hydrogels were prepared using a modification of a previously described technique.²⁵ The HEMA-PEG hydrogel solution was allowed to polymerize for 2 h at room temperature in 24-well tissue culture plates. After polymerization, hydrogels were swelled in phosphate buffered saline (PBS, Sigma-Aldrich), pH 7.4, overnight at room temperature. To elute cytotoxic components of the hydrogels, PBS soaking was continued for 72 h at room temperature.²⁵

Cell culture

Human monocytes/macrophages (THP-1, ATCC) were cultured in RPMI 1640 media supplemented with HEPES buffer (10 mM), sodium pyruvate (1 mM), glucose (4.5 g/L), fetal bovine serum (10%), penicillin (100 U/mL), streptomycin (100 µg/mL), and 2-mercaptoethanol (0.05 mM). Monocytes/macrophages were treated with 1 µg/mL LPS (Sigma-Aldrich) for 24 h, 50 nM PMA (Sigma-Aldrich) for 72 h, or left untreated.

Untreated and LPS-treated cells were resuspended in 2 mL fresh culture medium at a cell concentration of 500,000 cells/mL and seeded onto PE, PU, PMMA, ePTFE, ot-PVC, HEMA-PEG, or empty wells in a 24-well TCPS plate. The plates were incubated for 24 and 48 h. After 24 and 48 h, the cells were loosened from the materials by mechanical dissociation and the cells were collected. Cells were counted on a hemocytometer using a Nikon Diaphot light microscope (Nikon, Melville, NY). Live cells were determined via trypan blue dye exclusion. Supernatants collected at 24 and 48 h were stored at -20°C until analyzed for cytokine concentrations.

The PMA-treated cells were rinsed with PBS and detached with 4 mL of trypsin/EDTA (Clonetics, Walkersville, MD) per T-75 flask. The flask was incubated for 4 min before adding 8 mL of trypsin neutralizing solution (TNS; Clonetics). The cells were then re-suspended and seeded onto materials in the same manner as the untreated and LPS-treated cells. At 24 and 48 h postseeding the supernatants were collected and stored at -20°C. The cells were then loosened from the materials using 260 µL of trypsin/well, incubating for 4 min, and then adding 520 µL of TNS/well. Viable cells, determined by trypan blue dye exclusion, were counted on a hemocytometer via light microscopy.

Flow cytometry

In order to assess levels of monocyte differentiation, the expression of the cell surface marker CD11b, a marker of macrophage differentiation, was measured by flow cytometry. Untreated, LPS-treated, and PMA-treated cells were detached as described above and fixed for 15 min with 3.7% paraformaldehyde. The cells were then rinsed in PBS, incubated with 10% goat serum (blocking buffer; Sigma), and incubated with 10 µg/mL mouse anti-human CD11b (Becton Dickinson). Cells were again rinsed, then incubated with Alexa fluor 488 goat anti-mouse secondary antibody (Invitrogen), rinsed, and postfixed in 10% formalin. Fluorescence intensity of the labeled cells was measured using a FACSCalibur flow cytometer (Becton Dickinson). At least 10,000 events were acquired per sample to determine the geometric mean fluorescence intensity. Data analysis was performed using CellQuest software (Becton Dickinson).

Fluorescent imaging

Untreated monocytes/macrophages were seeded onto PE, PU, PMMA, ePTFE, ot-PVC, and TCPS surfaces in 24-well tissue culture plates at a seeding density of 500,000 cells/mL. The materials were rinsed with PBS to remove loosely adherent cells then incubated with 1 µM Calcein-AM (Sigma-Aldrich) for 15 min. Images of fluorescent cells were obtained via fluorescence microscopy using an Olympus BX41 (Olympus, Center Valley, PA) microscope.

Cytokine measurement

Supernatants collected from monocytes/macrophages seeded onto biomaterial surfaces were assayed for cytokines using a Luminex bead array (BioRad). Initially, a 27-plex human cytokine kit, which included TNF- α , MCP-1, MIP-1 α , IL-8, IL-1 β , IL-6, VEGF, IL-1ra, IL-10, IL-2, IL-4, IL-5, IL-7, IL-9, IL-12 (p70), IL-13, IL-15, IL-17, IFN- γ , IP-10, G-CSF, GM-CSF, RANTES, FGF-basic, Eotaxin, PDGF-bb, and MIP-1 β , was used to screen for potential cytokine targets (BioRad). Of this list of 27 cytokines, only the first nine were selected for continued analysis based on their measured concentrations (data not shown) and their roles in the foreign body reaction. Luminex bead array assays were performed according to the manufacturer's instructions (Linco/Millipore). Briefly, antibody coupled beads were incubated with monocyte/macrophage supernatants for 1 h. After washing, secondary antibody was incubated with the cytokine-bound beads for 30 min. Finally, phycoerythrin dye was bound to the beads via biotin-streptavidin binding for 30 min. All incubations occurred at room temperature on a plate shaker according to the manufacturers instructions. Sample concentrations (pg/mL) were determined from mean fluorescence intensities compared against a four- or five-parameter logistic standard curve generated from standards of known concentration provided by the bead array manufacturer. The Luminex bead array is calibrated prior to every sample analysis and validated regularly per the manufacturer's instructions.

Statistics

One-way ANOVA plus Bonferroni's multiple comparison test *post hoc* analysis was performed to determine significance in the flow cytometry data (GraphPad Prism 4). A multivariate analysis of variance (MANOVA) was determined from nine four-way ANOVAs (one per cytokine) to determine significance in the cytokine per cell and normalized cytokine data (StatView 5.0.1). *p*-values of <0.1, as determined by a multivariate test using Roy's Greatest Root, were considered significant for the material by treatment interaction term. Subsequently, *p*-values of <0.05 were considered significant in *post hoc* tests (Fisher's protected least square difference) used to compare between material effects.

RESULTS

Monocyte activation and differentiation

Human monocytes (THP-1) were treated with LPS, PMA, or left untreated. Phase contrast microscope images of these cells indicated that PMA-treated cells underwent a morphological change from untreated monocytes (Fig. 2). Whereas untreated cells were generally round and grew in clusters, PMA-treated cells exhibited larger, irregular shapes with extended filopodia and decreased cell clustering. LPS-treated monocytes were similar in morphology to untreated monocytes. Immunostaining, in combination with fluorescence activated cell sorting (FACS), was utilized to quantify the amount of expressed CD11b. FACS results showed a significant increase in CD11b expression for PMA-treated monocytes in comparison to untreated and LPS-treated monocytes.

Cytokine production per cell for monocytes/macrophages seeded onto biomaterials

From an initial screen of 27 human cytokines, 9 were selected for inclusion in this study based on concentration (>20 pg/mL) and their relevance to the foreign body reaction: TNF- α , MCP-1, MIP-1 α , IL-8, IL-1 β , IL-6, VEGF, IL-1ra, and IL-10 (data not shown). The raw data depicting cytokine concentrations for each combination of material, cell treatment, and time are ideal for precise concentrations; however, patterns in cytokine production are difficult to ascertain from a standard graph of this data (Appendix). To present the data in a manner more suitable for analyzing cytokine production patterns, these data were converted to a color map, similar to the familiar red-green gene expression grids. In addition, cytokine production was calculated on a per cell basis in order to account for variation in cell concentrations among the selected cell treatments and biomaterials. Cytokine production per cell was calculated by normalizing the cytokine concentrations measured from the supernatants of monocytes/macrophages seeded onto a material to their respective cell concentration. Cytokine production per cell, graphed on a color map using MATLAB (MathWorks, Natick, MA), is shown in Figure 3.

Cytokine production per cell generally increased from 24 to 48 h for all cytokines and all cell treatments. At both 24 and 48 h, cytokine production per cell was greater for PMA-treated cells than either untreated or LPS-treated cells for all cytokines, reaching as high as 100,000 ag/cell for three cytokines: MIP-1 α , IL-8, and IL-1ra. Patterns in material-induced cytokine production were difficult to distinguish; however, cytokine production induced in untreated cells by each of the four noncytotoxic biomaterials at 48 h was greater than that induced by TCPS.

Classification of total cytokine production

Because individual cytokine production can be difficult to interpret, we also looked at the integrated response of classes of cytokines. Total cytokine production is presented for all cytokines grouped by their pro and anti roles in inflammation and wound healing (Fig. 4). Untreated monocytes produced predominantly antiinflammatory and pro-wound healing cytokines on all surfaces, with the exception of ot-PVC. In contrast, for LPS- and PMA-treated monocytes, proinflammatory and anti-wound healing cytokines were produced in the greatest quantity. For all cell treatments, monocytes/macrophages seeded onto ot-PVC produced predominantly proinflammatory and anti-wound healing cytokines at both 24 and 48 h. It should also be noted that there was no apparent materials-dependent effect on class of cytokine produced among noncytotoxic materials.

Evaluation of TCPS “blank”

In order to examine whether TCPS was suitably benign to comprise a “blank,” we also examined cytokine production in response to a nonfouling HEMA-PEG hydrogel.²⁶ Cytokine production per cell for monocytes/macrophages seeded onto HEMA-PEG was normalized to cytokine production per cell for monocytes/macrophages seeded onto TCPS (Fig. 5). Of the 54 combinations in Figure 5, only in 11 cases was cytokine production induced by TCPS statistically different than HEMA-PEG. In eight of these cases, TCPS induced less cytokine production than HEMA-PEG. TCPS induced more cytokine

production than HEMA-PEG only for VEGF, a pro-wound healing cytokine, and IL-10, an anti-inflammatory cytokine.

Cell viability on material surfaces and material contact angles

After 24 and 48 h, viability of monocytes/macrophages seeded onto material surfaces was measured via trypan blue dye exclusion. Viability was always greater than 92% for LPS-treated and untreated cells seeded onto PE, PU, ePTFE, PMMA, or TCPS (Table I). Viability was much lower for PMA-treated cells, reaching as low as 29% for ot-PVC at 48 h. Viability for PMA-treated cells increased from 24 to 48 h for all materials except ot-PVC. Monocyte/macrophage viabilities were always the lowest for cells seeded onto ot-PVC, where monocytes/macrophages were 11% and 13–16% viable with no treatment and LPS-treatment, respectively. Static water contact angles measured on the selected biomaterials ranged from mildly hydrophilic 68° for TCPS to very hydrophobic 117° ePTFE (Table I).

Cell adhesion to material surfaces

At 24 h postseeding, untreated human monocytes/macrophages fluorescently labeled with 1 μ M calcein AM were imaged. Calcein AM fluorescence revealed that adherent cells were prevalent on PE, PMMA, ePTFE, PU, and TCPS (Fig. 6). Fluorescent images of ot-PVC surfaces showed substantially fewer viable, attached monocytes/macrophages than the other materials.

Cytokine production normalized to TCPS “blank”

In order to make comparisons between materials, cytokine production per cell induced by each material was normalized to cytokine production per cell induced by the TCPS blanks. Normalized cytokine production data were graphed on a color map using MATLAB (Fig. 7).

Normalized cytokine production was most frequently increased above the control (>1) for untreated monocytes. For untreated monocytes, few differences were noticed at 24 h; however, at 48 h, ot-PVC positive control induced significantly increased normalized production of TNF- α , MIP-1 α , and IL-8, which were all greater than 10-fold higher than TCPS. Normalized IL-1 α and IL-10 production were the lowest for ot-PVC, although these differences were not statistically significant. The only statistically significant differences between noncytotoxic biomaterials for untreated monocytes were increased normalized production of IL-10 induced by ePTFE as compared to PE or PU as well as increased VEGF induced by PMMA as compared to PU.

At 24 h, normalized cytokine production was not significantly increased for LPS-treated cells seeded onto ot-PVC in comparison to the noncytotoxic materials. At 48 h, normalized production values for TNF- α , MCP-1, and MIP-1 α were significantly higher for LPS-treated cells seeded onto ot-PVC as compared to other materials. No significant differences were noted between noncytotoxic biomaterials.

For PMA-treated cells, differences in normalized cytokine production with respect to material were not significant at 24 h. At 48 h, normalized cytokine production for PMA-treated cultures seeded onto PMMA and PE was increased for all cytokines, although these

increases were only statistically significant with MCP-1, IL-6, and IL-10 as compared to ot-PVC. Again, no statistically significant differences were noted between noncytotoxic biomaterials.

DISCUSSION

Macrophages at an implant surface are derived from monocytes that encounter molecular signals as they migrate into tissue from the vasculature. The arriving macrophages interrogate the implanted material and secrete an array of cytokines, chemokines, and growth factors to direct the foreign body reaction. Proinflammatory cytokines, such as TNF- α , IL-8, MIP-1 α , and MCP-1, recruit immune cells such as lymphocytes and monocytes to the implant site to promote inflammation. Antiinflammatory cytokines, such as IL-10 and IL-1ra, suppress proinflammatory signals in order to downregulate inflammation. Pro-wound healing cytokines such as VEGF play a reparative role by promoting neovascularization in the tissue surrounding the implant. While these classifications are useful in establishing general categories of cytokines, the specific functions of each individual cytokine are unique; thus, individual cytokine production levels are expected to vary for each cytokine within these classifications.

It is currently assumed that the types and concentrations of cytokines produced by macrophages determine the outcome of the foreign body reaction to a given implanted material.¹ The goals of this study were to examine the roles of (1) monocyte activation and differentiation into macrophages and (2) biomaterial surface chemistry on cytokine production.

An immortalized human monocyte line (THP-1) was selected for use in this study in order to minimize variability in cell populations. For our study, LPS and PMA mimicked the intrinsic activation and differentiation signals that monocytes encounter during the foreign body reaction as they migrate from the vasculature into tissues and become macrophages.²⁷ LPS simulates bacterial activation of macrophages, whereas PMA mimics the intracellular signaling functions of diacylglycerol.^{28,29} Previous research indicates that LPS and PMA each have a unique signaling pathway in monocytes, though both induce cytokine production through activation of the transcription factor NF- κ B.³⁰ Because of these unique pathways, cells treated with LPS and PMA exhibited different phenotypes. For instance, PMA induced a noticeable change in monocyte/macrophage morphology, differentiating these loosely adherent cells into an adherent state (Fig. 2). LPS-treated cells appeared morphologically similar to untreated cells. FACS indicated upregulation of the macrophage differentiation marker CD11b, a subunit of the CD11b/CD18 integrin involved in cell surface adhesion,³¹ after stimulation with PMA (Fig. 2). PMA induced the greatest increase in CD11b positive monocytes/macrophages, as evidenced by FACS and the increased spreading of monocytes/macrophages in Figure 2. Upregulation of CD11b and increased cell spreading suggested that PMA-treatment of monocytes/macrophages led to a greater number of cell-surface contacts and a phenotype more typical of macrophages, as compared to LPS treatment or no treatment.

The change in cell behavior as monocytes were activated or differentiated into macrophages was also apparent in the variation in cytokine production (Fig. 3). After just 24 h, PMA-treated macrophages produced an increased amount of cytokines when compared to LPS-treated and untreated cells (Fig. 3). This effect was even more apparent after 48 h. To a lesser degree, LPS-treatment increased proinflammatory cytokine production as compared to untreated monocytes. These results were not surprising since PMA and LPS alone are known to induce cytokine production from cell cultures.³⁰

Interestingly, when the cytokines were grouped for their roles in the foreign body reaction, it became apparent that monocyte activation or differentiation with LPS or PMA, respectively, preferentially induced proinflammatory and anti-wound healing cytokines (Fig. 4). These results suggest that uncommitted monocytes exhibit antiinflammatory and pro-wound healing profiles until they are activated or differentiated into macrophages, at which point they adopt a decidedly proinflammatory and anti-wound healing profile. Perhaps activated monocytes and macrophages, which are known to have a role in chronic inflammation,² are inherently more apt to promoting inflammation than unstimulated monocytes.

The interaction between macrophages and an implanted material is facilitated by a protein layer rapidly adsorbed to surfaces upon exposure to serum.² Since the conformation of proteins in this layer is dependent upon the surface chemistry of a biomaterial, it is postulated that the surface chemistry of a biomaterial could impact cellular functions including cytokine secretion. The primary goal of the current study was to investigate this possibility by analyzing the initial cytokine production, up to 48 h postseeding, from monocytes/macrophages interrogating bio-materials with varying surface chemistries. A 27-cytokine kit was used to identify cytokines and growth factors induced by monocyte/macrophage interaction with material surfaces. Seven cytokines found in greater than 20 pg/mL concentrations, TNF- α , MCP-1, MIP-1 α , IL-8, VEGF, IL-1 α , and IL-10, as well as the common proinflammatory cytokines IL-1 β and IL-6 were selected from the original 27 cytokines for examination in this experiment.

Although TCPS is commonly used for culturing cells, it is a surface foreign to monocytes/macrophages much like the other biomaterials. For this reason, TCPS was evaluated as a biomaterial “blank” in comparison to a nonfouling HEMA-PEG (HEMA-VP-PEG copolymer) hydrogel.^{25,26} The HEMA-PEG surface induced similar cytokine production to TCPS for all cell treatments (Fig. 5). Of the instances where TCPS and HEMA-PEG induced statistically significant differences in cytokine production, HEMA-PEG more often induced greater cytokine production than TCPS. The only instances where TCPS induced greater cytokine production than HEMA-PEG involved the antiinflammatory cytokine IL-10 and the pro-wound healing cytokine VEGF. In sum, as evaluated in comparison to a nonfouling hydrogel, TCPS is suitable as a “blank” for inducing cytokine production from monocytes/macrophages.

ot-PVC manufactured with an organo-tin stabilizer, a typical positive control in cytotoxicity testing,²⁴ was selected in this study as a positive control for inflammation. The cytotoxicity of ot-PVC was apparent in the first 24 h after cell seeding (Table I). The lack of viable cells adherent to ot-PVC could also be seen in the fluorescent images taken after calcein AM

staining (Fig. 6). In contrast, monocytes/macrophages were viable and adherent to the other five surfaces, including the background material, TCPS. Since significantly fewer cells survived in culture with ot-PVC, the amount of each cytokine produced would be expected to be lower as compared to other materials. To most accurately compare ot-PVC with the other materials, cytokine concentrations were calculated per concentration of viable cells present in culture with each material.

The positive control ot-PVC induced the greatest proinflammatory cytokine production by untreated and LPS-treated cells at 48 h (Fig. 3). The inflammatory effect of ot-PVC was even more apparent in the TCPS-normalized cytokine production, which was greater than 10 for the proinflammatory cytokines TNF- α , MCP-1, MIP-1 α , and IL-8 (Fig. 7). In addition, ot-PVC induced very little antiinflammatory or pro-wound healing cytokine production. ot-PVC was the only material to induce a proinflammatory and anti-wound healing cytokine profile for all cell treatments (Fig. 4). For this material, the cells were likely responding to the cytotoxic effect of organo-tin leached from the ot-PVC surface, rather than the ot-PVC surface itself. Nevertheless, ot-PVC depicted a cytokine profile representative of a material that induced high signaling for promoting inflammation and low signaling for promoting wound healing.

The common biomaterials PE, PU, ePTFE, and PMMA were selected because they are stable, chemically inert, and they possess a range of surface chemistries, as evidenced by the static water contact angles in Table I. Differences in cytokine production among these materials were most easily interpreted from the TCPS-normalized cytokine production data (Fig. 7). Normalized cytokine production values were greater than unity for all of these biomaterials. This suggests that the presence of these materials in TCPS culture plates increased cytokine production within the time frame of this study. In comparing materials, significant differences were rare among the noncytotoxic biomaterials. Differences in cytokine production due to the effects of the biomaterials were indistinguishable from the increased cytokine production induced by LPS and PMA. The only significant differences in normalized cytokine production between noncytotoxic biomaterials occurred with untreated cells (Fig. 7). Most notably, ePTFE induced significantly higher IL-10 production than PU or PE from untreated monocytes at 48 h. Since IL-10 is an antiinflammatory cytokine, this finding suggests that untreated monocytes cultured on ePTFE downregulate inflammation in comparison to those cultured on PE or PU. However, this result was not repeated for LPS- and PMA-treated cells. The only other significant difference between noncytotoxic biomaterials was increased VEGF production from untreated monocytes on PMMA as compared to PU. Again, this trend was not repeated for LPS-or PMA-treated macrophages.

When cytokines were categorized based on their role in the foreign body reaction, cytokine production from the noncytotoxic materials followed similar trends (Fig. 4). Generally, more total cytokine was produced at 48 h than at 24 h. In addition, while the total amount of cytokine varied, each noncytotoxic material induced similar patterns of pro- or antiinflammatory and pro- or anti-wound healing for all cell treatments. This suggests that these materials with variable surface chemistries might not promote substantially different immune responses *in vivo*, although these highly controlled *in vitro* conditions are not completely representative of an *in vivo* environment.

Although this study and previous research have shown cytokine production from monocytes/macrophages can differ with material surface,^{7,9,21} caution should be used in correlating individual cytokine production with material properties. With the exception of cytotoxic ot-PVC, differences in cytokine concentrations among biomaterials in this study were only moderate and not reflective of a shift in the promotion of inflammation or wound healing. Additionally, when monocytes were activated or differentiated into macrophages, no differences were apparent between these nontoxic biomaterials. Results from this investigation are consistent with the argument that, given time, the foreign body reaction resolves with the similar endpoint of fibrous encapsulation for nearly any commonly implanted material.¹⁷ While PE, PU, PMMA, and ePTFE are variable in surface chemistry, they are similar in being non-cytotoxic polymeric biomaterials; thus, protein and macrophage interactions may be similar for these surfaces. Nonspecific protein layers adsorb to these surfaces, regardless of surface chemistry, which may provide the signal that determines macrophage functions and, consequently, the foreign body reaction to implanted materials. While surface chemistry undoubtedly impacts protein adsorption, macrophages may respond to any nonspecific array of proteins in a similar fashion. Alternatives to manipulating implant surface chemistry include attaching cell or cytokine coatings to the surface of an implanted device, which could potentially direct the foreign body reaction through native signaling pathways. Ongoing research in our lab is aimed at investigating the potential of these coatings to favorably affect the immune response.^{32,33}

CONCLUSIONS

In this study, cytokine production was examined from resting monocytes, activated monocytes and differentiated macrophages seeded onto biomaterial surfaces. A new method of presentation was developed to illuminate patterns within cytokine production data. Cytokine production increased as monocytes were activated or differentiated into macrophages, with specific increases in proinflammatory and anti-wound healing cytokines, suggesting activated monocytes and differentiated macrophages were more prone to inducing inflammation than unstimulated monocytes. All biomaterials, including PE, PU, PMMA, and ePTFE, induced increased cytokine production in comparison with TCPS alone. With the exception of a known cytotoxic material, ot-PVC, the changes in cytokine responses with respect to material were not indicative of a substantial shift in the balance of inflammation and wound healing. The minor differences in induced cytokine production *in vitro* between these polymeric biomaterials, PE, PU, PMMA, and ePTFE, could potentially indicate similar foreign body reactions and fibrous encapsulations *in vivo*.

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APPENDIX

Figure A1.

Raw cytokine concentrations detected from monocytes/macrophages cultured on biomaterial surfaces are presented quantitatively. Data are shown as means $\pm$ SEM ($n = 3$).

Figure 1.

Cytokines have been characterized based on their roles in the foreign body reaction.³
Additions to the original source are italicized.

Figure 2.

Phase contrast images (20× magnification) of untreated, LPS-treated, and PMA-treated monocytes/macrophages. The geometric mean fluorescence intensities (GMFI) from FACS results of CD11b induction were graphed as geometric means + standard errors. *Geometric mean fluorescence intensity is statistically greater ($p < 0.05$) for PMA-treated cells than for untreated or LPS-treated cells.

Figure 3.

Cytokine production per cell (ag/cell) from monocytes/macrophages seeded onto biomaterial surfaces after (a) 24 h and (b) 48 h. A scale bar is included at the top of each color grid. Data are shown as means ($n = 3$). *Biomaterial-induced cytokine production is statistically greater ($p < 0.05$) than TCPS-induced cytokine production. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Figure 4.

Production of cytokines per cell (fg/cell) grouped into pro- or antiinflammatory and pro- or anti-wound healing based on each cytokine's role in the foreign body reaction. Data are shown as means $\pm$ SEM ($n = 3$).

Figure 5.

Cytokine production from monocytes/macrophages induced by HEMA-PEG hydrogels normalized to TCPS-induced cytokines. A scale bar is included above the color grid (normalized units). Data are shown as means ($n = 3$). Statistical significance is denoted as greater than (↑) or less than (↓) the TCPS blank ($p < 0.05$). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Figure 6.

Fluorescent images (10× magnification) of untreated monocytes/macrophages adherent to biomaterial surfaces. Calcein AM was used for fluorescent staining. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Figure 7.

Material-induced cytokine production normalized to TCPS-induced cytokine production for monocytes/macrophages seeded onto material surfaces for (a) 24 h and (b) 48 h. A scale bar is included above each color grid (normalized units). Data are shown as means ($n = 3$).

*Normalized cytokine production for ot-PVC is statistically greater ($p < 0.05$) than normalized cytokine production induced by other materials. Bars indicate statistical differences in normalized cytokine production between the connected biomaterials ($p < 0.05$). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Viability for Untreated, LPS Treated, and PMA Treated Monocytes/Macrophages at 24 and 48 h Postseeding onto Material Surfaces

TABLE I

Material (Contact Angle)	Untreated Viability (%)		LPS Treated Viability (%)		PMA Treated Viability (%)	
	24 h	48 h	24 h	48 h	24 h	48 h
PE (89° ± 2°)	99 ± 1	97 ± 1	98 ± 1	97 ± 1	55 ± 3	69 ± 4
PU (89° ± 1°)	99 ± 1	97 ± 1	99 ± 1	96 ± 1	50 ± 10	72 ± 9
ePTFE (117° ± 2°)	99 ± 1	98 ± 1	96 ± 1	96 ± 1	60 ± 10	74 ± 3
PMMA (73° ± 1°)	96 ± 1	97 ± 1	94 ± 1	93 ± 1	39 ± 6	77 ± 5
ot-PVC (69° ± 1°)	12 ± 1	11 ± 3	13 ± 4	16 ± 4	37 ± 7	29 ± 4
TCPS (68° ± 2°)	99 ± 1	98 ± 1	95 ± 1	97 ± 1	50 ± 20	75 ± 6

Static water contact angles are included for each material. Data are shown as mean ± SEM (*n* = 3).