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In Vivo Cytokine-Associated Responses to Biomaterials

RJ Schutte¹, L Xie¹, B Klitzman^{1,2}, and WM Reichert¹

¹Department of Biomedical Engineering, Duke University, Durham, NC 27708

²Kenan Plastic Surgery Labs, Duke University, Durham, NC 27708

Abstract

Cytokines, chemokines, and growth factors were analyzed periodically over eight weeks from the wound exudate fluid surrounding biomaterials implanted subcutaneously within stainless steel mesh cages. TNF- α , MCP-1, MIP-1 α , IL-2, IL-6, IL-1 β , VEGF, IL-4, and IL-10 were measured from exudate samples collected from cages containing specimens of polyethylene (PE), polyurethane (PU), or organo-tin polyvinyl chloride (ot-PVC). Empty cages served as negative controls, and lipopolysaccharide (LPS) served as a positive control. Cytokine, chemokine, and growth factor concentrations decreased from the time of implantation to eight weeks post-implantation, and there was an overall increase in cytokine, chemokine, and growth factor production for material-containing cages compared to empty cages. However, cytokine production was only modestly affected by the different surface chemistries of the three implanted polymeric materials.

INTRODUCTION

The functionality of implanted devices, particularly tissue-interactive devices, is dependent on the foreign body reaction that occurs in the tissue surrounding the implant [1]. A number of cells contribute to the foreign body reaction in vivo, including neutrophils, monocyte-derived macrophages, and lymphocytes. Cytokines and growth factors secreted by these cells are the molecular messengers that promote inflammatory events, such as neutrophil and macrophage recruitment, as well as wound healing events, such as collagen synthesis and neovascularization [2,3].

The specific signaling molecules produced by inflammatory cells that interrogate the surface of an implanted device have been hypothesized to influence the tissue response to an implanted biomaterial [4]. This study employed the cage implant system to assess the cells and cytokines/growth factors in the interstitial fluid that pools around biomaterials placed within the cage lumen. The list of factors included pro- and anti-inflammatory and pro- and anti-wound healing cytokines and growth factors to examine whether the inflammatory and wound healing character of the cage environment can be classified [5].

The cage implant system has been used extensively to investigate the in vivo immune response to biomaterials, including hydrogels, degradable scaffolds, nondegradable biomaterials, sensors and biological microelectromechanical systems [6–9]. Briefly, 4 cm long by 1 cm diameter cylinders of stainless steel mesh are implanted in rat, mouse, or rabbit subcutis to create a well-defined pocket into which biomaterials are implanted. Interstitial fluid

(“exudate”) may be extracted with a syringe and characterized for the cell and extracellular signaling protein content, including cytokines and growth factors.

Previously, the cage implant system was used to show that polyethylene (PE) induced a milder inflammatory reaction than an organotin impregnated poly(vinyl chloride) (ot-PVC)[10], which is recommended by the International Organization for Standardization as a positive control for cytotoxicity [11]. This assessment was based on elevated macrophage, alkaline phosphatase, and acid phosphatase concentrations; however, extracellular protein concentrations did not vary significantly between the PE and ot-PVC. Other cage implant studies have shown that specimen surface chemistry impacts macrophage adhesion, FBGC fusion, apoptosis, microvessel growth, and surface protein expression in vivo [12–16]. Of greatest relevance was the study by Brodbeck et al that used the cage implant system to show that material-adherent macrophages express mRNA for cytokines and growth factors such as IL-6, IL-8, IL-10, TGF- β , IL-1 β , and IL-13 in a surface chemistry dependent manner [5].

A small number of cytokine mRNA findings have been supported by assaying for the proteins that they encode [17,18]. Recently, we showed that varying the surface chemistry of stable, non-toxic, polymeric devices had only a modest impact on the broad range of cytokines produced by monocyte/macrophage cultures that interrogated polymer surfaces [19]. To our knowledge, the analogous in vivo experiment of quantifying the concentrations of a wide array of cytokines and growth factors produced at the surface of an implanted biomaterial has not been reported.

The goal of the current study was to investigate the in vivo production of cytokines in response to commonly implanted nondegradable polymeric biomaterials, specifically polyethylene (PE), polyurethane (PU), and organo-tin stabilized polyvinyl chloride (ot-PVC). Polymer specimens were placed within stainless steel mesh cages implanted subcutaneously in Sprague Dawley rats and the production of cytokines, chemokines, and growth factors in the exudate surrounding the implanted materials was examined periodically up to 8 weeks post-implantation. Results indicate that cytokine production was affected by the materials within the cage, but was only modestly affected by the chemistries of these three materials.

MATERIALS AND METHODS

Biomaterials

High-density polyethylene (PE, US Pharmacopeia, Rockville, MD), polyurethane/pellethane 2363-80AE-050824, a soft segment polyurethane based on polyether and aromatic isocyanate (PU, Polyzen, Inc., Apex, NC), and organo-tin stabilized polyvinyl chloride (ot-PVC, Smiths Medical, Hythe, UK) were cut into 1 cm² squares and sonicated for 20 minutes 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 minutes. These cleaned materials were then gas-sterilized with ethylene oxide and outgassed for 8 hours 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).

X-ray Photoelectron Spectroscopy (XPS)

XPS data were obtained using a Kratos Analytical Axis Ultra DLD electron spectrometer (Kratos Analytical Instruments, Manchester, UK). Monochromatic AlK α X-rays (1486.7 eV) were employed. Survey scans were generated using a 300 μ m by 700 μ m measurement area operating at 15 kV, 10 mA, and 150 W. Data were collected from 0 to 1200 eV in binding energy with 160 eV pass energy, a 1.0 eV step size, and a dwell time of 200ms per point. The

data acquisition and processing were done using CasaXPS software. The operation pressure of the spectrometer was typically in the 2×10^{-8} torr range with a system base pressure of 6×10^{-9} torr.

Cage Implant System

Stainless steel mesh (stainless steel type 316, 24×24 mesh per square inch, 0.014 inch wire diameter, MSC Industrial Supply Co., Melville, NY) was formed into cylindrical cages 1 cm in diameter and 4 cm in length. Protruding wire ends of the cages were turned inward. The cages were sterilized with ethylene oxide.

Male retired breeder rats (Sprague-Dawley) were anesthetized with isoflurane (~3% in oxygen, to effect) and their backs were shaved and skin prepped with chlorhexidine and alcohol scrub. Two 2-cm incisions were made approximately 1-cm lateral to the midline to expose the dorsal subcutis. Subcutaneous space was cleared by blunt dissection. One cage was implanted in the cleared space on each side and the incisions were closed in a single layer using interrupted monofilament cutaneous suture. In order to effectively isolate the tissue reaction to an implanted experimental biomaterial from the tissue reaction to the implanted cage, two weeks were allowed for progression of healing in the tissue surrounding the implanted cages. After two weeks, access to the cage lumen was made by incision and one PE, PU, or ot-PVC specimen was inserted within one of the stainless steel cages, while the other cage remained empty to serve as a control. Rats showing external signs of inflammation were immediately removed from the study.

As a positive control for inflammation, LPS (1 $\mu\text{g/g}$ rat body weight, Sigma) was injected into empty cages implanted into male retired breeder rats (Sprague-Dawley) for 24 hours. Exudate samples were collected as described below after 3 hours and 24 hours.

Exudate collection

Exudate samples were collected from empty and material-implanted cages on days 1, 2, 8, 28, and 56 after biomaterial implantation. Briefly, the rat was anesthetized with isoflurane (~3% in oxygen, to effect) and elevated to promote exudate fluid pooling at the posterior end of the cage. A 25 gauge, 1 inch needle was inserted through the skin into the posterior end of the cage lumen, and exudate was extracted slowly to prevent contamination with blood. Collected exudate was divided into two polypropylene containers on ice, one for cellular analysis and one for cytokine analysis.

Cell analysis

Cellular composition of the exudate fluid was analyzed by the Duke Veterinary Diagnostic Laboratory. Briefly, exudate fluid samples were smeared onto glass slides and stained using a Diff-Quick staining kit. Neutrophils, lymphocytes, monocytes, basophils, and eosinophils were counted on each slide using light microscopy (100x magnification) with standard clinical laboratory sampling protocols.

Cytokine measurement

Exudate fluid extracted from cages was assayed for cytokines with a Bioplex bead array (BioRad) using Lincoplex assay kits performed according to the manufacturer's instructions (Millipore). Briefly, biotinylated, antibody coupled beads were incubated with exudate fluid at 8°C for 18–24 hours. After washing, secondary antibody was incubated with the cytokine-bound beads at room temperature for 1 hour. The biotinylated beads were developed by incubation of phycoerythrin-streptavidin to at room temperature for 30 minutes. All incubations occurred on a plate shaker according to the manufacturers instructions. Sample

concentrations (pg/mL) were determined from mean fluorescence intensities (MFI) compared against a 4 or 5-parameter logistic standard curve generated from standards of known concentration provided by the bead array manufacturer. The Luminex bead array was calibrated prior to every sample analysis and validated regularly per the manufacturer's instructions.

Statistics

A multivariate analysis of variance (MANOVA) was determined from nine three way ANOVAs (one per cytokine) to determine significance in the cytokine concentration 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. Significance in the normalized cytokine data was determined by p-values of <0.05 measured using a two-way ANOVA. 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

Biomaterial surface characterization

Atomic compositions of the biomaterial surfaces (PE, PU, and ot-PVC) as characterized by XPS are shown in Table 1. The PE surface was composed of 91% carbon, 7% oxygen, and 2% nitrogen. The PU surface was composed of 83% carbon, 13% oxygen, and 4% nitrogen. The ot-PVC surface was composed of 78% carbon, 15% oxygen, 4% nitrogen, 2.3% chlorine and 0.3% tin. Other than confirming the basic surface composition of the polymers, XPS also showed the presence of tin in the ot-PVC.

Cellular composition in exudate fluid

Exudate fluid samples extracted from stainless steel mesh cages with and without an implanted biomaterial were analyzed for the composition of cells, including neutrophils, lymphocytes, monocytes, basophils, and eosinophils (Figure 1). At early time points, the exudate fluid collected from biomaterial-filled cages contained primarily neutrophils. As neutrophils decreased between day 2 and day 8, the quantity of lymphocytes increased and remained elevated from day 8 to day 56 in the extracted exudate fluid. Monocytes never accounted for greater than 20% of the cell population in the exudates. Furthermore, basophils and eosinophils combined for less than 1% of the total cell population (data not shown). This response is typical, and there was no significant effect of material presence or the surface properties on the types of infiltrating cells.

Cytokines in exudate surrounding implanted biomaterials

Cytokine content was measured from the extracted exudate fluid using a Luminex® bead array system. The nine cytokines measured from this exudate included TNF- α , MCP-1, MIP-1 α , IL-2, IL-6, IL-1 β , VEGF, IL-4, and IL-10. The concentrations of these cytokines in the extracted exudate fluid are presented in a colormap format, where magnitudes are represented by a color scale, in order to visualize patterns in cytokine production for the acute response (Figure 2) and chronic response (Figure 3).

On day 1 post implantation, there were twelve instances where cytokine concentrations were significantly different ($p < 0.05$) between cages containing PE, PU, ot-PVC, and empty cages (Figure 2a). Nine of twelve differences were due to increased cytokine production from material-containing cages in comparison to empty cages. The other three differences involved increased production of VEGF and IL-10, and decreased production of MCP-1 induced by PU

as compared to PE. On day 2 post implantation, significant differences were only detected for MCP-1, where PE induced a greater MCP-1 concentration than PU, ot-PVC, and the empty cage (Figure 2b). Cytokine concentrations decreased from days 1 and 2 to day 8 post implantation (Figure 2c). Whereas IL-6, VEGF, and IL-10 were all present at concentrations above 10,000 pg/mL during the initial 2 days, only IL-10 reached this concentration after 8 days. No statistically significant differences were observed on day 8.

Chronic cytokine concentrations on days 28 and 56 post implantation were lower than the levels detected after days 1, 2, and 8 (Figure 3). After 28 days, MCP-1, IL-6, and VEGF remained present at concentrations of 1,000 pg/mL or higher. Only MCP-1 and IL-6 were present above 900 pg/mL after 56 days. The only significant difference after 28 days involved IL-6 production, which was significantly increased for the PU implants as compared to the PE, ot-PVC and empty cages. No significant differences were detected between materials after 56 days.

LPS-induced cytokine production

As a positive control, cytokine concentrations were measured from exudate fluid extracted 3 and 24 hours after the empty cage was injected with LPS, which is known to induce an inflammatory reaction (Figure 4). By 3 hours, and continuing to 24 hours, LPS induced the production of TNF- α , MCP-1, MIP-1 α , IL-2, IL-6, IL-1 β , VEGF, IL-4, and IL-10. Of these, TNF- α , MCP-1, IL-6, IL-1 β , VEGF, and IL-10 were produced at the highest concentrations, all of which were greater than 200 pg/mL at both 3 and 24 hours. IL-4 was produced at the lowest concentration, with a maximum of 20 pg/mL after 24 hours.

Normalized cytokines from cage exudate

In order to examine the relative differences between materials, all cytokine concentrations were normalized to their respective concentration in the empty cages (Figure 5, Figure 6). The normalized acute data presented in Figure 5 show a general increase in cytokine production induced by the implanted materials in comparison to the empty cage. Twenty one out of a possible 27 material and cytokine combinations on day 1 were upregulated in comparison to the empty cage (greater than unity), although these differences were not statistically significant. In particular, TNF- α , MCP-1, MIP-1 α , IL-6, IL-1 β , VEGF, and IL-10 were greater than unity for at least two of the three materials tested. After 2 days, the implanted materials continued to induce upregulation of TNF- α , MCP-1, MIP-1 α , IL-6, IL-1 β , VEGF, and IL-10; however, the magnitude of this upregulation was decreased from day 1. After 8 days, the magnitude of material-induced cytokine upregulation decreased from day 2, although MIP-1 α , IL-6, IL-1 β , and IL-10 continued to be upregulated in comparison to the empty cage (Figure 5c).

Normalized chronic cytokine production after 28 and 56 days was similar, with MIP-1 α , IL-6, IL-1 β , IL-4, and IL-10 upregulated by implanted materials as compared to the empty cage, although this none of these differences were statistically significant (Figure 6). In particular, MIP-1 α and IL-6 were upregulated more than 10-fold for PE, PU, and ot-PVC as compared to the empty cages.

Integrated cytokine production

The integrated responses of classes of cytokines were examined by summing the cytokines produced based on their respective roles in the foreign body reaction (Figure 7). According to the 2 $\times$ 2 matrix of Brodbeck et al.[5], the cytokines detected in this study may be classified as follows: VEGF, IL-1 β , and IL-4 are pro-wound healing, IL-10, IL-2, IL-6, MCP-1, MIP-1 α , and TNF- α are anti-wound healing, IL-4 and IL-10 are anti-inflammatory, and IL-1 β , VEGF, IL-2, IL-6, MCP-1, MIP-1 α , and TNF- α are pro-inflammatory. For the material-implanted cages as well as the empty cages, the total amount of cytokine produced decreased after the

first two days. In all cases, the majority of cytokines detected were pro-inflammatory and anti-wound healing. While the materials induced an increased overall cytokine production during the first two days as compared to the empty cage, there was no apparent materials-dependent effect on the classes of cytokines produced. It is also interesting to note that the total cytokines detected during the acute phase from day 1 to day 8 were both pronounced and decidedly pro-inflammatory and anti-wound healing, whereas the chronic phase was characterized by a diminished but not distinguishably anti-inflammatory and pro-wound healing cytokine profile.

DISCUSSION

Macrophages, lymphocytes, and neutrophils are recruited to the site of implantation and react to the presence of a foreign material [3]. It is widely accepted that extracellular signals play a role in directing this foreign body reaction; however, these signaling processes are poorly understood. Until recently, in vivo studies that investigated the link between cell interrogation of materials and intercellular signaling relied on PCR to detect up or down regulation of cytokine and/or growth factor genes [4,5]. However, gene expression often exhibits a tenuous link to protein expression; whereas direct detection of proteins expressed by cells, either by ELISA or protein array, is less ambiguous.

Previously, we reported the direct in vitro profiling of cytokines and growth factors expressed in monocytes/macrophages cultured on common biomaterials [19]. The goal of this study was to perform the analogous in vivo cytokine profiling test by analyzing the infiltration of cells and the production of an array of signaling proteins, including cytokines and growth factors, from the exudate that pools around polymeric implant materials with different surface chemistries. In order to do so, a cage implant system was employed, whereby a material of interest was implanted subcutaneously within a stainless steel mesh cage, creating a pocket in the tissue for the collection of wound fluid. Samples were removed from this pocket, which was in direct contact with the surface of the implanted material. These samples were analyzed for the types of infiltrating cells and the concentrations of cytokines produced around the implanted material.

An analysis of the material surfaces using x-ray photoelectron spectroscopy (XPS) confirmed the presence of carbon, oxygen, and nitrogen in PE, PU, and ot-PVC (Table 1) [20,21]. Additionally, ot-PVC was shown to contain chlorine, a component of PVC, and tin, a component of the cytotoxic organotin stabilizer used in the manufacture of ot-PVC [22]. This organotin compound has been previously shown to have a cytotoxic effect on cell cultures and an inflammatory effect when implanted [10,23].

Fluid extracted from the cages was analyzed for neutrophils, lymphocytes, monocytes, basophils, and eosinophils (Figure 1). As expected from previous reports [3], neutrophils populated the site of implantation during the first two days, after which mostly lymphocytes and fewer monocytes migrated into this tissue. Basophils and eosinophils were an even smaller portion of the total cell population. There was no apparent affect of material surface property on the types of infiltrating cells.

The cytokine concentrations measured from the extracted exudates were as high as 10,000 pg/mL, specifically for IL-6, VEGF, and IL-10 (Figure 2, Figure 3). In contrast, TNF- α , MIP-1 α , and IL-4 were only detected in concentrations below 100pg/mL. Cytokine concentrations tended to decrease with time, although MCP-1 and IL-6 persisted at more than 900 pg/mL after 8 weeks of PE and PU implantation. The high concentration of IL-10 initially after implantation may have signalled the decrease in cytokine production observed at later time points, since IL-10 has been shown to downregulate cytokine production [24]. As expected with tissue undergoing trauma, IL-6 was present in the exudate, likely promoting inflammation

and pathogen clearance [25]. VEGF in the exudate fluid likely promoted the formation of vasculature to support the ingrowth of new tissue into the hollow cage[26]. MCP-1 production remained steadily near 1,000 pg/mL during the first 8 days, and decreased slightly after 28 and 56 days post-implantation. This relatively constant production of MCP-1 through 8 weeks of implantation was indicative of continuous monocyte recruitment to the site of material implantation. IL-2 concentrations also reached 1,000 pg/mL during the first 8 days of implantation, potentially inducing T-cells and B-cells to process antigens [27,28]. The presence of IL-1 β at the site of implantation also presumably served to fight against infection [29].

Of the twelve total significant differences detected on day 1, nine involved increases in cytokine production induced by material-containing cages as compared to empty cages. These data suggest that the presence of these polymer materials within the stainless steel cages had an impact on the intercellular signaling that occurs in the healing tissue. The remaining significant differences on day 1 were increased production of MCP-1, but decreased production of VEGF and IL-10 by PE as compared to PU. Based on the individual roles of these cytokines, these data would suggest that PE induces more pro inflammatory and less pro-wound healing signals than PU at this early time point. After two days, MCP-1 production was significantly increased in cages implanted with PE as compared to PU, ot-PVC, or empty cages. Again, this suggests that PE induces greater pro-inflammatory, anti-wound healing signaling than PU, ot-PVC, or empty cages. After 28 days, IL-6 production by cages containing PU was significantly increased as compared to cages containing PE, ot-PVC, or empty cages. No significant differences were found in the exudates collected after 56 days. The finding at 28 days suggests that PU induces a greater pro-inflammatory and anti-wound healing cytokine response than PE, ot-PVC, or empty cages, although the findings on days 1, 2, 8, and 56 do not support this trend.

Exudate from LPS-injected cages was examined as a positive control for inflammatory cytokine production (Figure 4). As soon as 3 hours, and up to 24 hours after LPS injection, cytokines were prevalent in the extracted exudate fluid. The cytokines induced by LPS were generally similar in magnitude to those induced by the implanted materials. However, LPS induced increased production of TNF- α in comparison to the implanted polymer materials, but decreased production of IL-2. This finding was not surprising, since LPS has been shown previously to strongly induce TNF- α , but only weakly induce IL-2 [30]. Despite these differences, the impact of LPS-injected cages on cytokine and growth factor production was largely similar to the impact of biomaterial-implanted cages.

All cytokine concentrations were normalized to the empty cages in order to isolate the effect of the material implanted within the cage (Figure 5, Figure 6). On days 1 and 2 post-implantation, the majority of material-cytokine combinations were upregulated as compared to the empty cage control, although this upregulation was not statistically significant. The upregulated cytokines included TNF- α , MIP-1 α , IL-6, IL-1 β , VEGF, and IL-10. The frequency and magnitude of this upregulation decreased from day 1 to day 8, but increased from day 8 to day 28 and day 56. The increase in normalized cytokine production after 28 and 56 days could be representative of a continued immune response to the implanted polymer materials, while the immune response to the empty cages is more resolved.

In order view the integrated response of cytokines with similar functions, cytokines were grouped into categories based on their roles in the foreign body reaction (Figure 7). From this figure, it was clear that the largest amount of cytokines, particularly for cages containing materials, was produced during the first two days after implantation, followed by a clear attenuation in the amount of protein detected; although there were still no significant shifts in the relative production of inflammatory and wound healing cytokines between the different implant materials, including cytotoxic ot-PVC. This integrated depiction of cytokine

concentrations also indicated that the majority of cytokines produced for all implanted cages were pro-inflammatory and anti-wound healing. However, it must be noted that the rat cytokine antibodies commercially available for measurement with the bead array were primarily pro-inflammatory and anti-wound healing, and only a few anti-inflammatory and pro-wound healing cytokines could be detected. This may be a technical explanation for why the expected transition from an acute pro-inflammatory and anti-wound healing environment to an anti-inflammatory and pro-wound healing chronic environment was not observed.

In previous in vitro studies we reported that PVC impregnated with cytotoxic organotin (ot-PVC) exhibited higher cytokine expression profiles than did organotin-free biomaterials [19]. However, the impact of this cytotoxic agent was not clearly exhibited in the current in vivo cytokine production study, even though the presence of the cytotoxic agent was confirmed by XPS analysis of ot-PVC. One possible explanation for this phenomenon was the inability to precisely control the exudate fluid sampled from within the implanted cage. Fluid aspirated from a subcutaneous pocket follows the path of least resistance, which is dependent on the architecture of the subcutaneous tissue [31]. Previous research indicates that this type of fluid sampling could extract fluid from tissue channels distant to the sampling point [32]. Since this fluid could be far from the biomaterial surface, its cytokine content could be independent of the implant material. Another possible explanation is that the immune response directed at the stainless steel mesh cage overwhelms the more subtle effect of the differences in material, masking any material-dependent effects on cell infiltration and cytokine production. One potential design improvement in the cage implant system would involve implanting cages composed of the material of interest, thereby eliminating the confounding effect of the large surface area of stainless steel mesh.

Similar to previous in vitro results, a small number of statistically significant differences were observed in cytokine production among the polymeric materials within the implanted cages, although these differences were not preserved over time [19]. When cytokine production was viewed in an integrated fashion based on the roles of individual cytokines in inflammation and wound healing, there were no significant shifts among the implanted materials. In light of these observations, we have been conservative in our interpretation of the differences in individual cytokine production observed in this study.

In this in vivo study, differences in cytokine and growth factor concentrations among biomaterials were only moderate and not reflective of a shift in the promotion of inflammation or wound healing. These results were 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 [33]. 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 [34]. Ongoing research aimed at investigating the potential of these coatings to favorably affect the immune response is promising [35,36].

CONCLUSIONS

In this study, the cage implant system was utilized in rat subcutaneous tissue to assess the impact of material surface chemistry on cytokine production and cell infiltration around the implant. Cytokine production was increased for cages containing PE, PU, and ot-PVC in comparison to empty cages, although there was no apparent effect on the types of infiltrating cells. The production of individual cytokines was, in a few instances, significantly different between these materials; however, when cytokines were grouped based on their roles in inflammation and wound healing, the cumulative production of these cytokine groups was not significantly affected by these materials. The chemical differences between these polymeric

materials did not result in significant changes in the cytokine profiles in the surrounding tissue. The similar cytokine profiles induced by these materials suggest that similar tissue reactions, such as tissue vascularization and fibrous tissue formation, would be seen in vivo for these chemically dissimilar, polymeric biomaterials.

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Figure 1. Exudate cellular composition

a) Neutrophils, b) lymphocytes and c) monocytes extracted from PE, PU, and ot-PVC filled cages, as well as empty cages. Data are shown as means $\pm$ SEM ($n \geq 3$).

Figure 2. Acute in vivo cytokine concentrations (day 1, day 2, day 8)

Cytokine concentrations (pg/mL) in the exudate extracted from the tissue inside an implanted cage on a) day 1, b) day 2 and c) day 8. A scale bar is included at the top of each color grid. Data are shown as means ($n \geq 4$). *Bars indicate statistically significant differences in cytokine concentrations ($p < 0.05$) between materials.

Figure 3. Chronic in vivo cytokine concentrations (day 28, day 56)

Cytokine concentrations (pg/mL) in the exudate extracted from the tissue inside an implanted cage on a) day 28 and b) day 56. A scale bar is included at the top of each color grid. Data are shown as means ($n \geq 4$). *Bars indicate statistically significant differences in cytokine concentrations ($p < 0.05$) between materials.

Figure 4. In vivo LPS-induced cytokines

Cytokine concentrations (pg/mL) in the exudate extracted from the tissue inside an implanted cage treated with LPS after 3 hours and 24 hours. A scale bar is included at the top of the color grid. Data are shown as means (n = 3).

Figure 5. Acute in vivo normalized cytokines (day 1, day 2, day 8)

Normalized cytokine production (normalized units) from exudate extracted from the tissue inside an implanted cage on a) day 1, b) day 2 and c) day 8. A scale bar is included at the top of each color grid. Data are shown as means ($n \geq 4$).

Figure 6. Chronic in vivo normalized cytokines (day 28, day 56)

Normalized cytokine production (normalized units) from exudate extracted from the tissue inside an implanted cage on a) day 28 and b) day 56. A scale bar is included at the top of each color grid. Data are shown as means ($n \geq 4$).

Figure 7. In vivo integrated cytokine production

Cytokine concentrations (ng/mL) grouped into pro- or anti-inflammatory and pro- or anti-wound healing based on each cytokine's role in the foreign body reaction. Data are shown as means $\pm$ SEM ($n \geq 4$).

Table 1

Atomic composition of polymers

The elemental compositions of PE, PU, and ot-PVC were determined by XPS.

	C	O	N	Cl	Sn
PE	91%	7%	2%	-	-
PU	83%	13%	4%	-	-
ot-PVC	78%	15%	4%	2%	<1%