Final Draft
of the original manuscript:

Roch, T.; Schulz, C.; Jung, F.; Ma, N.; Lendlein, A.:
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In: Clinical Hemorheology and Microcirculation (2014) IOS Press

DOI: 10.3233/CH-141826

Interaction of poly(ether imide) films with early immune mechanisms

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Abstract: With the worldwide increase of atherosclerosis, the need for new engineered patient specific implants such as stents or vascular grafts is still emerging. Recently, very smooth poly(ether imide) (PEI) films were, based on their excellent hemocompatibility and compatibility with endothelial cells, suggested as potential biomaterial for cardiovascular applications. In atherosclerosis, immune mechanisms such as complement activation, but also cellular responses such as monocytes and neutrophils activation, can mediate the inflammatory response. Therefore, it is important that the implant material itself does not trigger the inflammatory response. Early immune mechanisms - e.g. macrophage activation, complement induction, generation of reactive oxygen species (ROS), and the secretion of inflammatory cytokines by leukocytes - could potentiate the inflammatory responses, and may thereby alter endothelial cells behaviour or facilitate platelet activation. Therefore, it is important to evaluate the immuno-compatibility of PEI-films.
The PEI-films were fabricated from commercially available PEI, which was dissolved in dichloromethane and pulled out on a cleaned, smooth glass surface and subsequently, solvent residues were removed during the drying procedure.
Using a murine macrophage reporter cell line possible material bound microbial contaminations and material intrinsic immuno-stimulatory properties were investigated. The macrophages were viable after adhering on the PEI-films and did not show signs of activation, indicating that the used PEI-film was free of microbial contaminations. To determine whether PEI-films induced complement activation, the release of C5a in pooled human plasma was analyzed. The detected C5a levels did not differ between PEI-films and tissue culture plates (TCP), which served as control material. Furthermore, in whole human blood, the generation of ROS as well as the cytokine production were investigated by flow cytometry and by multiplex bead arrays, respectively. The production of IL-6 and TNF-α as well as the generation of ROS by immune cells of the whole blood was not induced upon contact with PEI-films.
The immunological evaluation of PEI-films revealed that no substantial activation of the investigated early immune mechanisms occurred. Altogether, this data demonstrate that PEI is immuno-compatible and from that perspective may be a suitable biomaterial for cardiovascular applications.

Keywords: Immuno-compatibility, endotoxins, biomaterial, poly(ether imide), cardiovascular implants

1. Introduction

Non-communicable diseases like cancer or cardiovascular diseases are the major course of death in high-, middle-, and lower-income countries [8]. Although the cardiovascular death rates are slightly declining in most countries, vascular occlusion is still a major cause of death requiring the need for new engineered patient specific implants such as stents or vascular grafts particularly to treat small caliber blood vessels occlusions like in coronary arteries [8,26]. Polymer-based cardiovascular implants have successfully been established, but several properties of the next generation of cardiovascular implants could only be fulfilled by multifunctional polymers [14]. The *in vivo* performance of implants is strongly dependent on surface properties such as topography, charge, wettability but also on the chemical composition [24]. Additionally, for cardiovascular implants, hemocompatibility and compatibility with endothelial cells are essential features [17].

Recently, a very low platelet adhesion required for a good hemocompatibility was shown on poly(ether imide) (PEI) films, most likely because of their smooth surface (R_q = 3.2 ± 1.5 nm measured in wet state) [7]. Additionally, endothelial cells, which form the inner layer of blood vessels providing thereby a hemocompatible surface, could also adhere to the PEI-films and showed a similar cellular behavior and morphology as on TCP [25]. The low thrombogenicity, the compatibility with endothelial cells, and the processability qualifies the PEI-film as candidate material for cardiovascular applications. However, besides compatibility with region-specific cells, biomaterials have to be immuno-compatible in order to avoid unwanted immune activation, which can be associated with severe adverse effects for the patient [17]. Pyrogenic contaminations of biomaterials including endotoxins and other microbial products can lead to unspecific immune reactions characterized by the activation of immune cells such as macrophages or dendritic cells and their release of inflammatory mediators [10]. The cellular response of particular immune cells towards microbial products is mediated by engagement of toll-like receptors (TLR), which are evolutionary conserved pattern recognition receptors [27]. Beside the immunogenic effects of pyrogenic contaminations, also cells whose primary functions are not related to immune reactions can directly be influenced by microbial products. For example, endothelial cells and thrombocytes can be activated by TLR4, the receptor for endotoxins, which results in the production of inflammatory mediators [5,19].

However, inflammatory processes can also be induced by intrinsic biomaterial properties, which may ultimately result in a foreign body reaction [3]. For example, relatively rough surfaces, as found on the outer surfaces of expanded poly(tetrafluoroethylene) vascular

prostheses, facilitated a foreign body reaction, which was characterized by adhesion of macrophages and foreign body giant cells [2]. The induction of innate immune mechanisms such as induction of complement, activation of macrophages, or generation of ROS by biomaterials are determining the immunological short- and long-term behavior [1]. Ideally, cardiovascular implant materials behave immunological inert or even have immune-suppressive capacities [10]. The scope of this study was to determine the level of microbial contaminations, which is essential for future investigations. Furthermore, since the immunogenic potential of PEI-films is not known so far, the capacity of the PEI-films to activate innate immune mechanisms should be analyzed.

By using cell-based assays, it was firstly investigated whether PEI-films contain microbial contaminations. In the second step, the PEI-films were evaluated for their inherent immunological properties including their capacity to induce complement activation, ROS induction, and cytokine response in whole human blood.

2. Material and Methods

2.1. Preparation of poly(ether imide) films (PEI-films)

PEI-films with a thickness of 300 µm were prepared as previously described [7,25]. Briefly, commercially available PEI (ULTEM® 1000, General Electric, New York, NY, USA) was solved in dichloromethane (DCM) and pulled out on smooth glass surface. PEI-films were dried under steadily increasing temperature (up to 105 °C) and decreasing atmospheric pressure (down to 15 mbar) for 27 days. Prior to use, the PEI-films were sterilized by steam sterilization for 20 min at 121°C and 200 kPa using a FVA A1 autoclave (FEDEGARI, INTEGRA Biosciences). The content of remaining solvents was determined by headspace gas chromatography (HP 7694 Headspace-Sampler, HP 5890 Series II GC, DB-624, 75m column) as previously described [25]. The contact angle and surface topography of the PEI-film were determined as previously described by contact angle measurements and atomic force microscopy, respectively [25].

2.2 Activation and viability determination of reporter cells

The RAW-Blue™ activation assay was performed as previously described and the HEK-Blue™-hTLR4 activation assay was adapted accordingly [31]. Briefly, $5x10^5$ RAW-Blue™ cells or HEK-Blue™-hTLR4 (InvivoGen, San Diego, USA) were cultured directly in 1 mL VLE-RPMI (Biochrom®) in the presence of the PEI-film for 24 hours. After incubation, cell culture supernatants were harvested for QuantiBlue™ analysis, the cell morphology was

evaluated by phase contrast microscopy and the cell viability assessment was performed using fluorescein diacetate (FDA, 25 $\mu g \cdot mL^{-1}$) and propidium iodide (PI, 2 $\mu g \cdot mL^{-1}$) (both Sigma Aldrich) [23]. Each sample was evaluated at three different fields of view using a confocal laser scanning microscope (LSM 510 META, Zeiss) with the AxioVision (Zeiss) image analysis software.

2.3 C5a activation assay

To detect the release of C5a 300 µL of pooled normal human serum (Precision BioLogic Inc. Dartmouth, Nova Scotia, Canada) were added to the PEI-film or TCP (Tissue Culture Test Plates, TPP, Trasadingen, Switzerland) as control and incubated for 30 minutes at 37 °C. As positive control, 100 $\mu g \cdot mL^{-1}$ zymosan (Sigma-Aldrich, St Lois, MO, USA) were used. A commercial enzyme-linked immunosorbent assay (ELISA) kit (R&D, Minneapolis, MI, USA) was used according to manufacture instructions to determine the amount of released C5a fragments in plasma, which was diluted 1:100 in DPBS (Life Technologies GmbH**,** Frankfurt/Main, Germany).

2.4 Whole blood sampling and cytokine detection

According to the criteria of the Nordkem-workshop [6,13], blood was obtained from apparently healthy volunteers. The study protocol received an approval by the institutional committee of the Charité Universitätsmedizin Berlin, Germany.

Blood was taken from the uncongested arm after puncture of the cubital vein and collected in Monovettes (10 mL, Sarstedt), each containing heparin as anticoagulant**.** The experiments were started immediately after collecting the blood sample.

To analyse the cytokine secretion profile, the whole human blood was diluted 1:10 in serum free VLE-RPMI (Biochrom®, Germany) and incubated for four hours in the presence of PEI-films or on TCP. Lipopolysaccharide (LPS) from the *E.coli* strain O111:B4 (Sigma-Alrich, Germany) was used as positive control. After incubation, the samples were centrifuged at 600 g for 7 min and supernatants were harvested. The Bioplex® 200 (Biorad, Munich, Germany) multiplex system was used to analyse the secretion pattern of IL-1β, IL-6, and TNF-α. The Bio-Plex® assay was performed according to manufacture instructions.

2.5 Phagoburst assay

Whole human blood was incubated for 10 min with or without PEI-films. Opsonised *E. coli* provided with the Phagoburst® assay kit (ORPEGEN Pharma, Heidelberg, Germany) were

used as positive control. After incubation, the Phagoburst® assay was performed according to manufacture instructions. The evaluation of oxidative burst activity was performed by flow cytometry using the MACSQuant® analyzer. Flow cytometry data were analysed using FlowJo software (Tree Star, Ashland, USA).

3. Results and Discussion

3.1 Determination of contaminations with microbial products

According to the U.S. Food and Drug Administration, eluates of implant materials should not exceed LPS levels above 0.5 EU·mL^{-1}, unless the devices gets in contact with cerebrospinal fluid where the limit is then set to 0.06 EU·mL^{-1} [12]. Using the *Limulus amebocyte* lysate (LAL)-test, we could previously show that the LPS levels in eluates prepared according to ISO10993-12 were below 0.06 EU·mL^{-1} [25]. Since the LAL-test only detects soluble endotoxins, a cell-based test system was established to additionally detect material-bound endotoxins, which could, due to the chemical structure of LPS, strongly adsorb to the hydrophobic PEI-film surfaces [12,25].

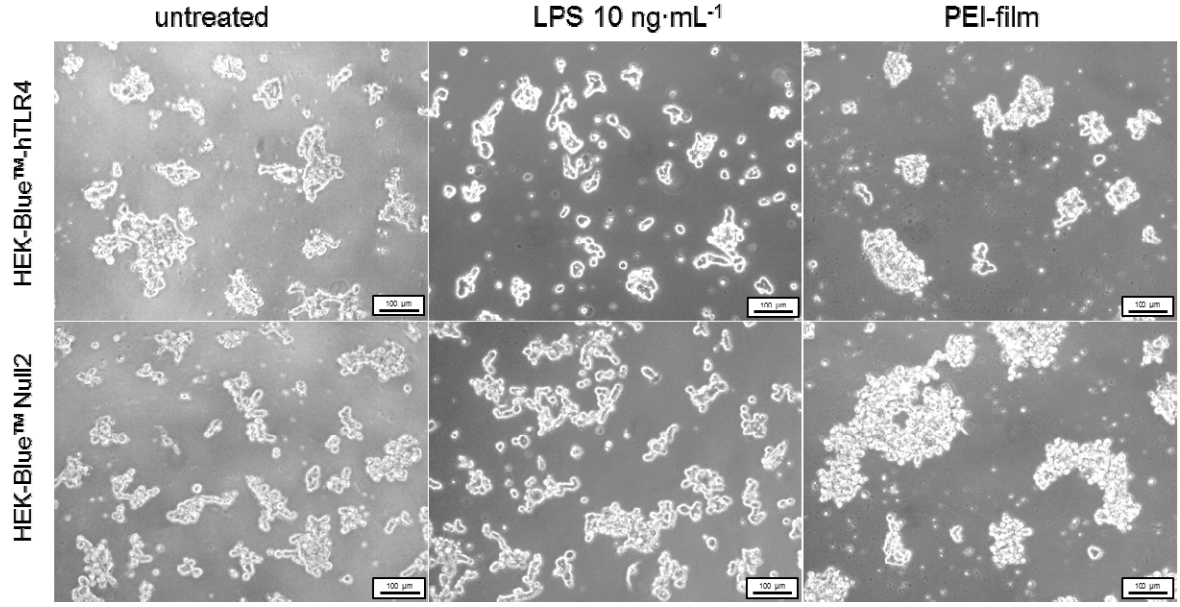

Figure 1. HEK-Blue^{TM}-hTLR4 cells cultured on PEI-films to detect material bound endotoxins. Microscopic images of HEK-Blue^{TM}-hTLR4 cells (top) and HEK-Blue^{TM}Null2 control cells (bottom), which were cultured for 24 hours on TCP without stimulation (left panel), stimulated with 10 ng·mL^{-1} LPS (middle panel), or cultured on PEI-films (right panel).

The presence of material-bound LPS was analyzed using HEK-BlueTM-Null2 and HEK-BlueTM-hTLR4 cells by cultivating them for 24 hours on the PEI-films. No morphological alterations could be observed when compared to cells cultured on TCP either with or without LPS (Figure 1). After TLR4 engagement, the HEK-Blue^{TM}-hTLR4 cells can secrete an

embryonic alkaline phosphatase (AP), which is proportional to amount of LPS contamination and can be quantified by using QUANTI-Blue™ medium. The HEK-Blue™-Null2 cells serve as control since they bear the same genetic modifications allowing the secretion of AP, but lack the capacity to express TLR4. Thus, only HEK-Blue™-hTLR4 cells, but not HEK-Blue™-Null2 cells, can be activated by LPS (Figure 2). When both cells types were cultivated for 24 hours on PEI-films, neither of them secreted AP indicating that the material surfaces were not contaminated with LPS.

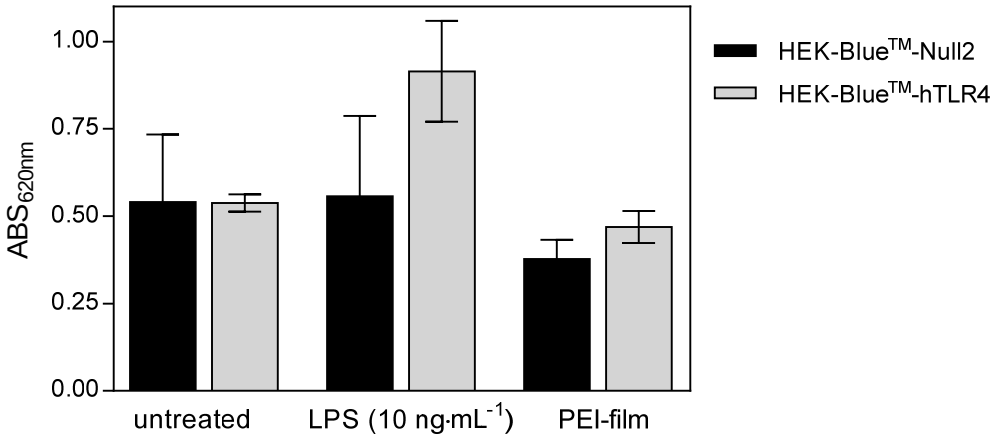

Figure 2. Detection of soluble and material-bound LPS using HEK-Blue™-hTLR4 cells. Supernatants form HEK-Blue™-hTLR4 cells and HEK-Blue™Null2 control cells cultured for 24 hour under the condition indicated in the figure were incubated for three hours with Quanti-Blue™ solution to detect released AP. The average of two individual experiments each analysed in triplicates is shown. Error bars indicate mean ± standard deviation (SD).

However, in addition to LPS, also other microbial products for example derived from fungi or Gram-positive bacteria may contaminate biomaterials resulting in similar immunological consequences as an LPS burden [21,29]. The presence of material bound microbial products was investigated using RAW-Blue™ cells, which express a broad spectrum of TLRs, whose activation leads to the secretion of AP detectable by the QUANTI-Blue™ assay. RAW-Blue™ cells adhered to the PEI-films, which did not influence their viability after cultivation for 24 hours as demonstrated by FDA/PI staining (Figure 3).

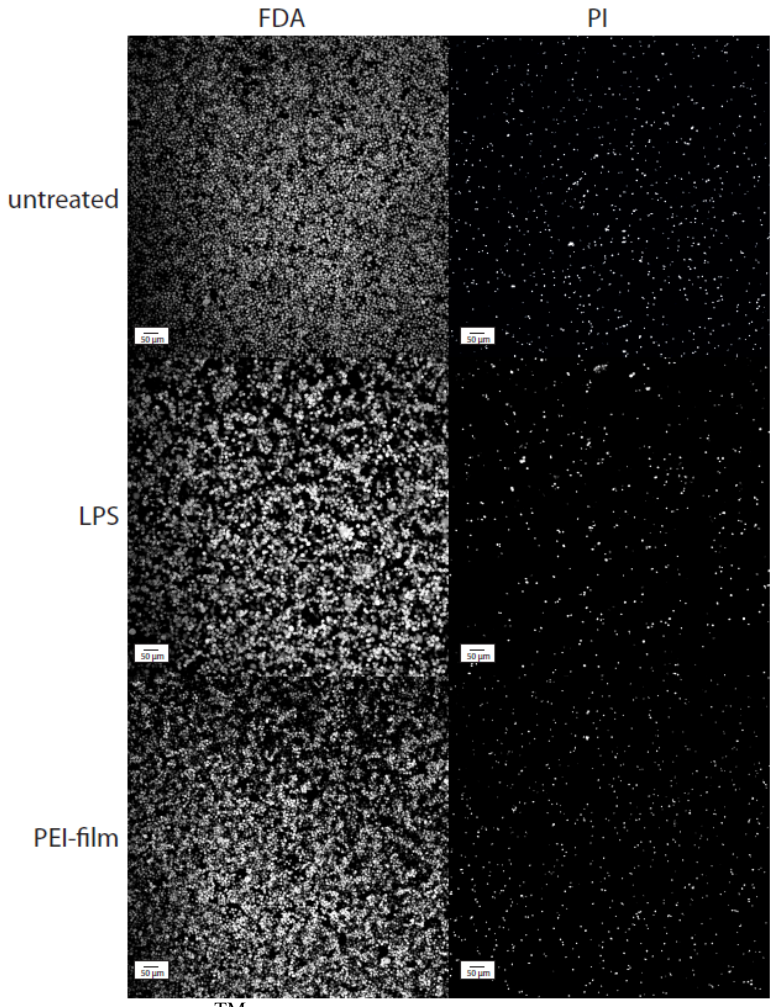

Figure 3. Survival of RAW-Blue^{TM} cells cultured on PEI-films. Viability was determined by staining with FDA (live cells) and PI (dead cells). Representative images of RAW-Blue^{TM} cells cultured for 24 hour without stimulation (top), activated with 10 ng·mL^{-1} LPS (middle) or in the presence of the PEI-films (bottom).

RAW-Blue^{TM} cells can be activated by LPS resulting in AP release catalyzing the conversion of the QUANTI-Blue^{TM} substrat into a colored dye (Figure 4). When the RAW-Blue^{TM} cells were cultivated on the PEI-film, they did not show signs of activation (Figure 4) suggesting that the PEI-films were not contaminated with microbial products. In agreement with the results obtained from HEK-Blue^{TM}-hTLR4 cell experiments, it can be concluded that the PEI-films are free of LPS and other materials-bound immune-stimulatory microbial products.

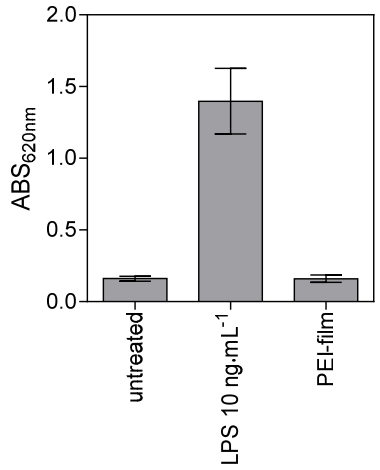

Figure 4. Detection of soluble and material-bound microbial contaminations using RAW-Blue™ cells. Blue™ cells cultured for 24 hour under the condition indicated in the figure were incubated for three hours with Quanti-Blue™ solution to detect the amount of released AP. The average of two individual experiments each analysed in triplicates is shown. Error bars indicate mean ± SD.

3.2 Analysis of reactive oxygen species induced by PEI-films

Biomaterials can induce ROS in particular cells of the immune system such as monocytes and neutrophils. While low concentration of ROS may induce proliferation, migration, and apoptosis of endothelial cells, elevated levels could induce inflammatory reactions [15]. Furthermore, ROS can react with polymeric biomaterials accelerating their degradation, which could subsequently lead to an impaired or completely lost implant functionality [16]. The production of ROS by immune cells in response to biomaterials can be tested in whole human blood [23]. When the blood was incubated with heat killed *E. coli*, a high frequency of ROS-producing monocytes and neutrophils could be detected (Figure 5).

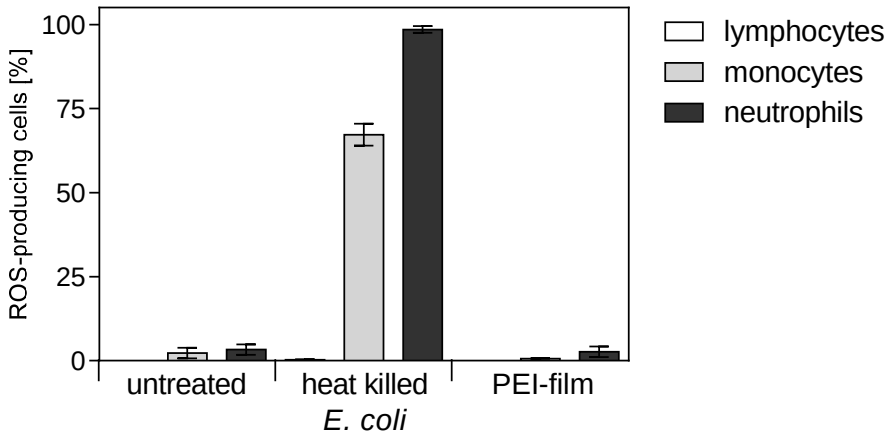

Figure 5. Effect of the PEI-film on production of ROS by whole blood-derived immune cells. Whole human blood was incubated for 10 minutes on TCP with and without heat killed *E.coli* (positive control) and in the presence of the PEI-film. The percentage of ROS-producing cells was determined by conversion of dihydrorhodamine-123 into rhodamin-123 and analyzed by

flow cytometry. Lymphocytes, neutrophils, and monocytes were discriminated by their flow cytometric scatter parameters as previously described [23]. The average values of four apparently healthy human donors are shown (mean ± SD).

However, unstimulated blood as well as blood incubated in the presence of PEI-films did not show elevated levels of ROS-producing monocytes and neutrophils. As expected, lymphocytes did not produce ROS under any of the tested conditions (Figure 5). The absence of ROS-producing cells after contact of blood with the PEI-films supports a future evaluation of this biomaterial for applications in cardivascular devices.

3.3 The influence of PEI-films on the cytokine production of whole human blood

Cytokines released by immune cells can act immuno-stimulatory or immuno-suppressive. In the context of cardiovascular implants, the device itself should not induce the release of pro-inflammatory mediators, because they can directly influence the behaviour of platelets or endothelial cells [18,19]. For example, IL-1β treatment led to higher uptake rates of nanoparticles by endothelial cells [30]. Pro-inflammatory mediators can also support local and systemic inflammatory reaction and attract immune cells to the side of implantation [14]. Furthermore, released cytokines can support the activation of platelets, which would increase the risk of thrombotic events after device implantation [22]. Therefore, it is essential for the performance of vascular implants that they do not induce inflammatory responses in whole blood. In order to investigate the capacity of the PEI-film to induce cytokine secretion in blood, the PEI-film was incubated for four hours with fresh human blood diluted in cell culture medium. The secretion of the pro-inflammatory cytokines IL-1β, IL-6, and TNF-α was determined (Figure 6). While LPS induced strong cytokine response, no substantial IL-1β, IL-6, and TNF-α production could be observed after incubating the blood with the PEI-films (Figure 6). Other cytokines such as IL-2, IL-4, IL-10, IL-12, IL-17, and IFN-γ could neither be detected after LPS stimulation nor after incubating the blood in the presence of PEI-films (data not shown). Altogether, the data indicate that the PEI-films did not induce cytokine release in whole human blood. This is in agreement with recently published data showing that PEI-films did not induce IL-6 and IL-8 responses by endothelial cells [25].

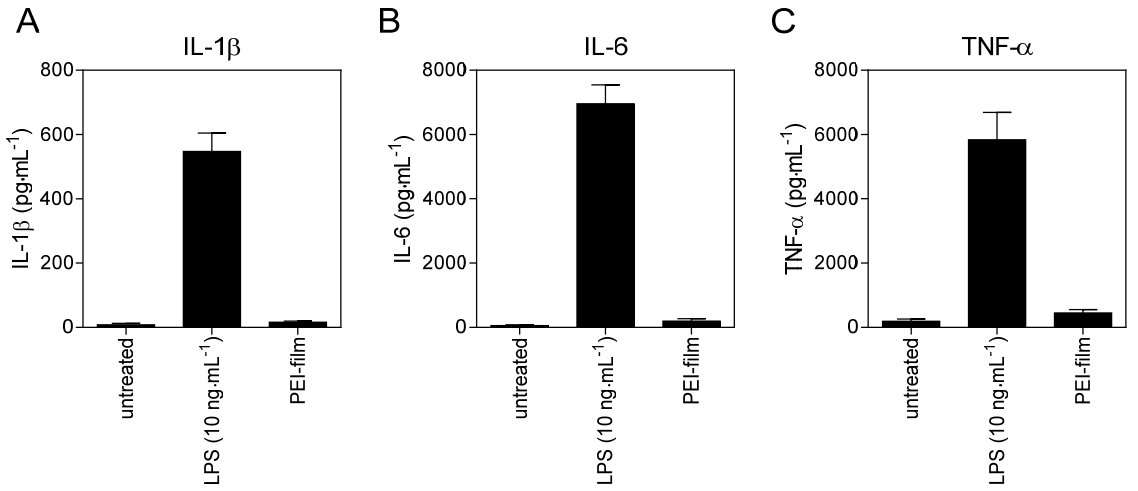

Figure 6. Cytokine production in whole human blood after incubation with the PEI-film. Anti-coagulated whole human blood was diluted 1:10 and incubated as indicated. The secretion of (A) IL-1β, (B) IL-6, and (C) TNF-α was determined by Bio-Plex®. The average cytokine expression from three healthy donors is shown (mean ± SEM).

3.4 Complement activation influenced by inserts with different roughness levels

In order to analyse the complement activation possibly induced by the PEI-films, the generation of C5a in human plasma was determined after incubation with the PEI-films. C5a release can be induced by microbial products such as zymosan, a compound derived from the yeast cell wall [20]. When pooled human plasma was incubated with zymosan, C5a was formed in a dose dependent manner (Figure 7). After incubating the plasma with the PEI-film, no significant induction of C5a could be observed. Additionally, the zymosan induced C5a release was not affected by the PEI-film, indicating that the PEI-film should not influence physiological complement activation (Figure 7). The induction of C5a by biomaterial surfaces is thought to be mediated by C3, which become enzymatically active due to the conformational changes induced by biomaterial surfaces [4]. Complement activating biomaterial surfaces are usually characterized by nucleophilic end groups such as hydroxyl or amino groups, whereas negatively charged groups such as carboxyl and sulfate, sialic acid, and bound heparin appear to be non-activating [11,28]. Furthermore, the materials hydrophobicity and hydrophilicity can affect the complement cascade, at which moderately hydrophobic surfaces appear to be more prone to induce complement activation [9]. According to contact angle measurements, the PEI-films are considered to be hydrophobic, (θ_{adv} = 85.3° ± 2.1°) and do not bear nucleophilic end groups [25]. Hence, the results showing that the PEI-film did not induce complement activation were expected and are in agreement with the literature.

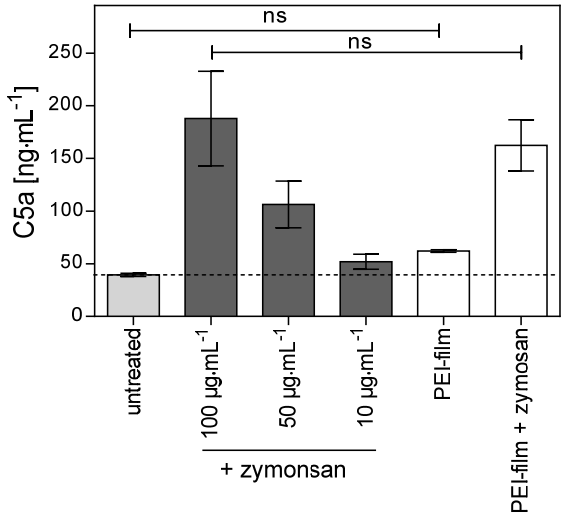

Figure 7. Complement activation in the presence of the different PEI and PS inserts. Pooled human serum was incubated for 30 min in PS and PEI inserts. C5a release was determined by ELISA. Data shown are pooled from three independent experiments each performed with in triplicates. For statistical analysis One-way ANOVA test with a Tukey posttest for multiple comparisons was performed (ns = not significant; mean ± SD).

4. Conclusion

As results of a very controlled and a largely germ free manufacturing process, it could be shown that the PEI-films used in this study were free of endotoxins and other microbial products. This prerequisite is essential for studying material intrinsic immunological effects or material-induced alterations of cells and tissues. The subsequent immunological evaluation revealed that the PEI films are compatible with different innate immune mechanisms. In detail, the PEI-films did not induce the production of ROS in blood derived human neutrophils and monocytes. Moreover, neither the complement cascade nor the release of inflammatory cytokines was induced. Conclusively, our data demonstrate that PEI-films are immuno-compatible and may therefore be a suitable biomaterial candidate for cardiovascular and other *in vivo* applications.

Acknowledgment

The authors thank Ruth Hesse, Anja Müller-Heyn, and Angelika Ritschel for the technical support and the German Federal Ministry of Education and Research (BMBF) for funding within project number 0315696A „Poly4Bio BB“.

References

[1] J.M. Anderson, Inflammatory response to implants, *ASAIO Trans* **34** (1988), 101-7.

[2] J.M. Anderson, BIOLOGICAL RESPONSES TO MATERIALS, *Annual Review of Materials Research* **31** (2001), 81-110.

[3] J.M. Anderson, A. Rodriguez and D.T. Chang, Foreign body reaction to biomaterials, *Semin Immunol* **20** (2008), 86-100.

[4] J. Andersson, K.N. Ekdahl, R. Larsson, U.R. Nilsson and B. Nilsson, C3 adsorbed to a polymer surface can form an initiating alternative pathway convertase, *J Immunol* **168** (2002), 5786-91.

[5] R. Aslam, E.R. Speck, M. Kim, A.R. Crow, K.W. Bang, F.P. Nestel, H. Ni, A.H. Lazarus, J. Freedman and J.W. Semple, Platelet Toll-like receptor expression modulates lipopolysaccharide-induced thrombocytopenia and tumor necrosis factor-alpha production in vivo, *Blood* **107** (2006), 637-41.

[6] B. Berg, H. Solberg, J. Nilsson and N. Tryding, In: Solberg H, Grasbeck R, Alstrom T (ed) Reference Values in Laboratory Medicine, *John Wiley & Sons Chichester (UK)* (1981), 55–64.

[7] S. Braune, M. Lange, K. Richau, K. Lützow, T. Weigel, F. Jung and A. Lendlein, Interaction of thrombocytes with poly(ether imide): The influence of processing, *Clinical Hemorheology and Microcirculation* **46** (2010), 239-250.

[8] D. Butler, UN targets top killers, *Nature* **477** (2011), 260-1.

[9] K.N. Ekdahl, B. Nilsson, C.G. Gölander, H. Elwing, B. Lassen and U.R. Nilsson, Complement Activation on Radio Frequency Plasma Modified Polystyrene Surfaces, *Journal of Colloid and Interface Science* **158** (1993), 121-128.

[10] S. Franz, S. Rammelt, D. Scharnweber and J.C. Simon, Immune responses to implants - a review of the implications for the design of immunomodulatory biomaterials, *Biomaterials* **32** (2011), 6692-709.

[11] M.B. Gorbet and M.V. Sefton, Biomaterial-associated thrombosis: roles of coagulation factors, complement, platelets and leukocytes, *Biomaterials* **25** (2004), 5681-703.

[12] M.B. Gorbet and M.V. Sefton, Endotoxin: the uninvited guest, *Biomaterials* **26** (2005), 6811-7.

[13] R. Gräsbeck, T. Alström and N. workshop, *Reference values in laboratory medicine : the current state of the art*, Wiley, Chichester.

[14] F. Jung, C. Wischke and A. Lendlein, Degradable, Multifunctional Cardiovascular Implants: Challenges and Hurdles, *MRS Bulletin* **35** (2010), 607-613.

[15] K.N. Kader and C.H. Coyle, Reactive oxygen and nitrogen species: implications for cardiovascular device engineering, *J Biomed Mater Res B Appl Biomater* **83** (2007), 138-44.

[16] M. Lange, S. Braune, K. Luetzow, K. Richau, N. Scharnagl, M. Weinhart, A.T. Neffe, F. Jung, R. Haag and A. Lendlein, Surface functionalization of poly(ether imide) membranes with linear, methylated oligoglycerols for reducing thrombogenicity, *Macromol Rapid Commun* **33** (2012), 1487-92.

[17] A. Lendlein, A.T. Neffe, B.F. Pierce and J. Vienken, Why are so few degradable polymeric biomaterials currently established in clinical applications?, *International Journal of Artificial Organs* **34** (2011), 71-75.

[18] J.A. Lumadue, S.M. Lanzkron, S.D. Kennedy, D.T. Kuhl and T.S. Kickler, Cytokine induction of platelet activation, *Am J Clin Pathol* **106** (1996), 795-8.

[19] V. Mako, J. Czucz, Z. Weiszhar, E. Herczenik, J. Matko, Z. Prohaszka and L. Cervenak, Proinflammatory activation pattern of human umbilical vein endothelial cells induced by IL-1beta, TNF-alpha, and LPS, *Cytometry A* **77** (2010), 962-70.

[20] S. Nagata and M.M. Glovsky, Activation of human serum complement with allergens. I. Generation of C3a, C4a, and C5a and induction of human neutrophil aggregation, *J Allergy Clin Immunol* **80** (1987), 24-32.
[21] S.M. Opal, Endotoxins and other sepsis triggers, *Contrib Nephrol* **167** (2010), 14-24.
[22] S.M. Opal and C.T. Esmon, Bench-to-bedside review: functional relationships between coagulation and the innate immune response and their respective roles in the pathogenesis of sepsis, *Crit Care* **7** (2003), 23-38.
[23] T. Roch, A. Kruger, K. Kratz, N. Ma, F. Jung and A. Lendlein, Immunological evaluation of polystyrene and poly(ether imide) cell culture inserts with different roughness, *Clin Hemorheol Microcirc* **52** (2012), 375-89.
[24] N. Scharnagl, S. Lee, B. Hiebl, A. Sisson and A. Lendlein, Design principles for polymers as substratum for adherent cells, *Journal of Materials Chemistry* **20** (2010), 8789-8802.
[25] C. Schulz, M. von Rüsten-Lange, A. Krüger, A. Lendlein and F. Jung, Viability and function of primary human endothelial cells on smooth poly(ether imide) films, *Clinical Hemorheology and Microcirculation* **52** (2012), 267-282.
[26] D.G. Seifu, A. Purnama, K. Mequanint and D. Mantovani, Small-diameter vascular tissue engineering, *Nat Rev Cardiol* **10** (2013), 410-421.
[27] O. Takeuchi and S. Akira, Pattern recognition receptors and inflammation, *Cell* **140** (2010), 805-20.
[28] M. Toda, T. Kitazawa, I. Hirata, Y. Hirano and H. Iwata, Complement activation on surfaces carrying amino groups, *Biomaterials* **29** (2008), 407-17.
[29] Vijayakumar R., Sandle T. and Manoharan C., A review on fungal contaminations in pharmaceutical products and phenotypic identifications of contamunats by convetional methods, *European Journal of Parenteral & Pharmaceutical Science* **17** (2012), 4-18.
[30] C. Wischke, A. Kruger, T. Roch, B.F. Pierce, W. Li, F. Jung and A. Lendlein, Endothelial cell response to (co)polymer nanoparticles depending on the inflammatory environment and comonomer ratio, *Eur J Pharm Biopharm* **84** (2013), 288-96.
[31] C. Wischke, S. Mathew, T. Roch, M. Frentsch and A. Lendlein, Potential of NOD receptor ligands as immunomodulators in particulate vaccine carriers, *J Control Release* **164** (2012), 299-306.