POLSKI
PRZEGLĄD CHIRURGICZNY
2015, 87, 9, 443–452

10.1515/pjs-2015-0086

DE GRUYTER
OPEN

EFFECTS OF GELATINE-COATED VASCULAR GRAFTS ON HUMAN NEUTROPHILS

FRANK MEYER[1], THOMAS BUERGER[2], ZUHIR HALLOUL[3], HANS LIPPERT[4], BRIGITTE KÖNIG[5], JOERG TAUTENHAHN[6]

Department of General, Abdominal and Vascular Surgery, University Hospital, Magdeburg[1]
Department of Vascular Surgery, Municipal Hospital "Diako", Kassel[2]
Division of Vascular Surgery, Department of General, Abdominal and Vascular Surgery, University Hospital, Magdeburg[3]
Institute of Quality Assurance, Otto von Guericke-University, Magdeburg[4]
Institute of Medical Microbiology, University Hospital, Leipzig[5]
Department of Vascular Surgery, Municipal Hospital, Magdeburg, Germany[6]

The aim of the study was to investigate the immune-modulatory potential of commercially available PTFE and polyester vascular grafts with and without gelatine-coating. The biomaterial-cell-interaction was characterized by changes of established parameters such as PMN-related receptors/mediators, phagocytosis potential and capacity as well as the effect of an additional plasma-dependent modulation.

Material and methods. By means of a standardized experimental in vitro model, various vascular graft material (PTFE/polyester/uncoated/gelatine-coated) was used for incubation with or without plasma and co-culturing with human neutrophile granulocytes (PMN) followed by analysis of representative receptors and mediators (CD62L, CD11b, CXCR2, fMLP-R, IL-8, Elastase, LTB_4). Oxidative burst assessed phagocytosis capacity.

Results. Comparing the vascular grafts, un-coated PTFE induced the lowest magnitude of cell stimulation whereas in case of gelatine-coating, cell response exceeded those of the other vascular grafts. This was also found comparing the polyester-based prosthetic material. Gelatine-coated polyester led to a more pronounced release of elastase than gelatine- coated PTFE and the uncoated materials. The results of oxidative burst indicated a reduced phagocytosis capacity in case of gelatine-coated polyester. Plasma incubation did also provide an impact on the cellular response. While in case of gelatine-coating, PMN-related receptor stimulation became lower, it increased by native polyester. The latter one did also induce more mediators such as IL-8 and LTB_4 than gelatine-coated material.

Conclusions. There have been no extensive data on cell-cell interactions, cytokines and general histo-/hemocompatibility of human cells by the new generation of vascular grafts. It remains still open whether healing process and infectious resistance can be compromised by material-dependent overstimulation or reduced phagocytosis potential of the immune cells of the primary unspecific immune response induced by gelatine-coated materials.

Key words: biocompatibility, gelatine, vascular grafts, human neutrophils

Vascular prostheses manufactured from polyester (Polyethylenterephthalate) or PTFE (Polytetrafluorethylen) can be considered the mostly used alloplastic materials in vascular surgery. Both synthetic materials differ in chemical structure and manufacturing. To improve biocompability and healing rate, to reduce thrombogenic potential, to avoid bleedings through the prosthetic wall, and to increase the infectious resistance, various coatings such as albumin, collagen, gelatine, carbon or silver were tested (1, 2).

Each implantation leads to an inflammatory response, mainly caused by the surgical trauma and the biomaterial-tissue interaction, which involves cellular and molecular equivalents of the specific and unspecific immune

response (3, 4). Polymorphonuclear neutrophilic granulocytes (PMN) are the strongest portion of effector cells in acute inflammatory processes, which are also basicly involved in the healing process. This cell fraction represents a great part of the unspecific immune response and mediates coupling to the specific immune defence, which is reflected in the preferred use for compatibility testing (5-8).

As far as known from the accessible literature, the effect of gelatine-coating on human neutrophils has not been investigated yet. Therefore, the aim of the study was to investigate the immunmodulatory characteristics of commercially available PTFE and polyester prostheses without and with gelatine-coating using a standardized experimental in vitro model. In this regard, we analyzed the i) neutrophil adhesion potential (characterized by CD62L, CD11), ii) signalling responses to chemoattractants (CXCR2, f-MLP receptor), iii) inflammatory mediator release (IL-8, elastase, leukotriene B4), and iv) parameters of microbicidal activity (oxidative burst). In addition, the role of plasma-coating for receptor expression was addressed.

MATERIAL AND METHODS

Purification of human neutrophils

Human peripheral mononuclear cells (PMN) were isolated from heparinized (VACUTAINER®, 7 mL; Natriumheparinat, 15 U/mL; Becton Dickinson GmbH, Heidelberg, Germany) peripheral blood obtained from healthy donors (n=10) using Polymorph-prep® (NYCOMED PHARMA AS, Oslo, Norway) according to the manufacturer´s description and standard protocol. The purified cell fraction contained more than 95 % of pure PMN as determined with a hematocytometer (Micro Diff II®, Coulter Electronics GmbH, Krefeld, Germany). The viability was 99 % as revealed with trypan blue exclusion. The PMN were diluted in phosphate buffered saline (PBS) to a final concentration of 2 x 10^6 cells per mL.

Activation of human blood neutrophils

The following commercially available vascular grafts were used: Uncoated (P1) and gelatine-coated (P2) PTFE (P1 = GORE-TEX® Vascular Graft; W.L. Gore & Associates Inc., Flagstaff/Arizona, USA; P2 = VASCUTEK TERUMO SEALPTFE□/taperflow□; Vaskutek Limited, Inchinnan/Refrewshire, Scotland, UK) in juxtaposition to uncoated (P3) and gelatine-coated (P4) polyester (P3 = MICRON KNITTED□/MICRON RADIALLY SUPPORTED KNITTED™, Intervascular®, La Ciotat Cedex, France; P4 = VASCUTEK TERUMO Gelsoft Plus™; Vaskutek Limited, Inchinnan/Refrewshire, Scotland, UK). Vascular grafts of defined size (diameter, 6 mm; length, 5 mm) were left untreated or were pretreated with plasma of the individual healthy blood donors at 37°C for 30 min. Thereafter, PMN (in total, 2 x 10^6) were added to a final volume of 1 mL (Safe-Lock 1.5-mL reaction tube; Eppendorf, Germany) and incubation proceeded at 37°C under 5 % of CO_2 for 2 h. All cell pellets were analyzed for surface molecules (CD62L, CD11, fMLP-receptor, CXCR2) as well as for burst activity. The respective supernatants only from stimulated grafts with no previous preincubation with plasma were taken for the analysis of leukotriene B4, IL-8, and neutrophil elastase.

Flow cytometry

The surface phenotypes of the cells were examined with flow cytometry (FACSCalibur®, Becton Dickinson Biosciences, Heidelberg, Germany) using murine monoclonal antibodies (Becton Dickinson, unless otherwise specified) to human leukocyte cluster of differentiation (CD) and other designated antigens. The specificities of monoclonal antibodies were as follows: CD11b (Mac-1), L-selectin (Leu-8), f-MLP-R, CXCR2. The antibodies were conjugated to fluorescein isothiocyanate (FITC) or phycoerythrin (PE). Appropriate isotype controls and electronic gating were used in each experiment to eliminate non-specific binding of murine immunoglobulins to these human leukocytes.

Mediator release

IL-8 was determined by IL-8 specific ELISA (DPC Diagnostic Products Corporation, Los Angeles (CA), USA) using the IMMULITE®

instrument (DPC Biermann GmbH, Bad Nauheim, Germany). PMN elastase was determined by turbometry (Hitachi Automatic Analyzer® 704; Hitachi Ltd., Tokyo, Japan) with an Elastase-PMN Immunoassay (Merck KG, Darmstadt, Germany). Leukotriene B_4 (LTB_4) was analysed using an LTB_4 Immunoassay (R&D Systems Inc., Minneapolis, USA). All experiments were performed in triplicates.

Respiratory burst

Oxidative burst was measured quantitatively by fluorometric analysis in human neutrophils using commercial methods (PHAGOBURST®; Orpegen Pharma, Heidelberg, Germany). The analyses were performed within 4 h of sampling.

Statistical analysis

The statistical evaluation has been performed with SPSS (version, 14.0) for Windows®. With regard to receptor expression, data were expressed as means ± SEM obtained and calculated from two different experiments. Data from mediator release were expressed in SI-units. The means between different treatments were compared by means of variance analysis as appropriate.

Study was undertaken in accordance with the Declaration of Helskinki for Biomedical Research and the recommendations and guidelines of the Institutional Review Board.

RESULTS

Adhesion molecules

CD62L. The first step of the multi-step adhesion cascade is the rolling of the leucocytes, which is characterized by a decrease in surface CD62L in vivo (shedding). This process is self-limited to allow further continuation of the cascade. A possible reduction of the CD62L expression represents a reliable marker for cell activation and can be used to compare the different prosthetic material.

The greatest reduction of CD62L were found in gelatine-coated PTFE prosthesis followed by the gelatine-coated polyester prosthesis (P2>P4>P3>P1; P2 versus P1/P3/P4, p = 0.00). The differences between gelatine-coated prosthesis and the respective uncoated prosthesis were significant (P2 to P1/P3, p=0.00; P4 to P1/P3, p=0.00). Differences between both uncoated prosthesis (P1, P3) were not seen. The lowest values were observed in case of ePTFE (P1).

The pretreatment with plasma changed the effects on CD62L expression especially on prosthesis P1, P2, P3 (P1 with ("+") versus without ("-") plasma (PL), P = 0.03; P2 ± PL, p = 0.00; P3 ± PL, p = 0.00). In this regard, the decrease in PMN-dependent CD62L expression upon interaction with gelatine-coated grafts (P2, P4) was less as compared to experiments in the absence of plasma. Nonetheless, the difference in CD62L expression between the gelatine-coated PTFE prosthesis and the gelatine-coated polyester prosthesis still remained. The effects of the gelatine-coated PTFE prosthesis (P2) was still significantly different to the respective uncoated basic materials (P1, P3) (P2 versus P1 and P3, p = 0.03 and p = 0.00, respectively). In the presence of plasma, a significant difference became apparent between both uncoated basic materials (P3 versus P1, p= 0.00).

CD11b. In continuation of the adhesion cascade, the CD18/CD11b receptor (Mac-1) plays a substantial role, e.g., cell-to-matrix and cell-to-cell interactions are coordinated by multiple receptor-ligand bindings. Cell stimulation induces an upregulation of the receptor. The extent of this increased expression is used as a parameter to compare the prosthetic material.

Analysis of the increase in CD11b expression with no plasma treatment showed the same pattern of median values of box plots as found in CD62L shedding, namely, P2>P4>P3>P1. Significant differences could be obtained between the gelatine-coated PTFE prosthesis (P2) and the prosthesis P1, P3, and P4 (P2 versus P1, P3, P4, p = 0.00, p = 0.00 and p = 0.01, respectively). Uncoated ePTFE generated the lowest increase of CD11b expression with significant differences to the remaining prosthetic materials (P1 versus P3 and P4, P = 0.00).

Pretreatment of the prostheses with plasma resulted in a significant reduction of CD11b expression in gelatine-coated PTFE (P2 ± PL,

p = 0.04; P3>P2>P4>P1). Thus, a significant difference between gelatine-coated and uncoated prosthesis was no longer observed. A significant difference in CD11b upregulation was only obtained between the uncoated polyester prosthesis and the uncoated ePTFE (P3 versus P1, p = 0.01) (fig. 1).

Chemokine receptors

CXCR2, a chemokine receptor (IL-8RB), is important for chemotaxis, cell activation (degranulation) and adhesion cascade of the PMN, which possesses a high affinity to IL-8 as well as to other chemokines. Since the reexpression of the receptor occurs distinctly later (within hours) compared with CXCR1 (within minutes), decrease of CXCR2 was used as parameter to compare the prosthetic materials under the standardized experimental conditions.

The CXCR2 expression profile with no in vitro plasma pretreatment was very similar as reported for CD62L and CD11b indicated by the median values of the box plots: P2>P4>P3>P1. The decrease of CXCR2 expression in P2 was significantly different to the prostheses P1, 3 and 4 (P2 versus P1, P3 and P4, p = 0.00, p = 0.0 and p = 0.01, respectively). Gelatine-coated polyester also generated a significantly different CXCR2 expression compared with native materials such as PTFE/polyester (P4 versus P1 and P3, p = 0.04 and 0.03).

Including plasma pretreatment in vitro, differences of CXCR2 expression for P2 and P4 were significantly lower compared with the respective values in the absence of plasma (P2 ± PL, p = 0.00; P4 ± PL, p = 0.04). Although the gelatine-coated PTFE prosthesis still showed the most pronounced effects on CXCR2 expression (P2>P4>P1>P3), a significant difference was only observed between the gelatine-coated PTFE prosthesis and the uncoated one (P2 versus P1, p = 0.04). Among the remaining prosthetic materials, no significant differences were found in the presence of plasma.

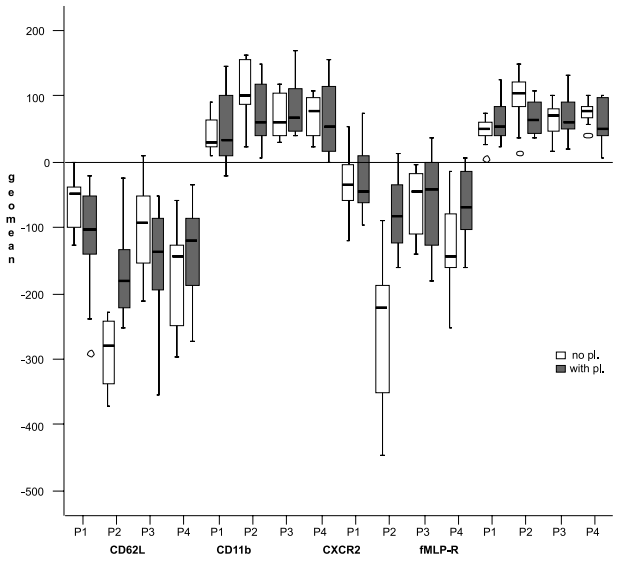

Fig. 1. Receptor expression on human neutrophils. Changes of receptor expression on human neutrophils after interaction with uncoated (P1, P3) and gelatine-coated (P2, P4) vascular grafts are shown. Experiments were performed in the absence (no pl) and presence of human plasma (with pl). Data were calculated by substracting the geomean channel fluorescence of the unstimulated neutrophils from that of neutrophils exposed to the devices. Experiments were performed in triplicates and data were obtained from at least 8 different experiments

fMLP receptor

Signal transduction via specific fMLP receptors induces various types of inflammatory response, which is accompanied by the induction of chemotaxis, release of lysosomal enzymes, generation of toxic oxygenic radicals, rearrangement of the cytoskeleton and initiation of phagocytosis. The changes of the receptor expression as possible stimulation effect of the prostheses onto the PMN served as parameter for comparison.

Gelatine-coated PTFE and polyester prostheses generated a significantly more pronounced effect on fMLP upregulation in comparison to uncoated material (P2>P4>P3>P1; P2 versus P1/P3, p = 0.00 and p = 0.02, respectively; P4 versus P1/P3, p = 0.00 and p = 0.04, respectively). Again, the lowest values were found for uncoated ePTFE prosthesis. In the presence of plasma, there was a significant reduction of the receptor expression for the gelatine-coated prostheses P2 and P4 (P2 ± PL, p = 0.04; P4 ± PL, p = 0.04) resulting in no detectable differences among all prostheses under study (fig. 1).

Inflammatory mediator release

With regard to the inflammatory mediator release, there was a different activation pattern by the various prosthetic materials as compared to their modulatory effects on surface molecules (fig. 2).

PMN are capable to generate and release a variety of proinflammatory molecules (IL-1, IL-6, IL-8 and TNFα). Among them, ***IL-8*** has an impact on various cell functions via G-protein associated receptors such as CXCR1 and CXCR2. The most pronounced stimulatory effects on IL-8 release from human PMN was induced by the uncoated polyester (P3) and uncoated PTFE prostheses (P1) (P3>P1>P4>P2). A significant difference was found in comparison to the gelatine-coated PTFE prosthesis (P3 versus P2, $p = 0.04$; P1 versus P2, $p = 0.03$). Gelatine-coated polyester prosthesis differed also significantly to the gelatine-coated PTFE material (P4 versus P2, $p = 0.00$).

Human neutrophil ***elastase*** release represents a marker of cell reactivity. Comparing the various prosthetic materials, gelatine-coated polyester prosthesis (P4) induced the highest elastase release from human neutrophils with significant difference versus P1, P2 and P3 (P4 versus P1, P2 and P3, $p = 0.00$, $p = 0.04$ and $p = 0.00$, respectively). In addition, gelatine-coated PTFE material was different to the uncoated material (P2 versus P1, $p = 0.02$). The uncoated prosthesis were the weakest inducer for elastase release from neutrophils (P4>P2>P3>P1).

Leukotriens are responsible for a strong chemotactic activity as well as for proinflammatory effects such as the expression of surface molecules for adhesion and transmigration, degranulation and tissue destruction. The highest LTB_4 values were observed after incubation of PMN with polyester showing a significant difference to the gelatine-coated polyester and gelatine-coated/uncoated PTFE prostheses (P3>P2>P4>P1; P3 versus P1, P2 and P4, $p = 0.00$, $p = 0.00$ and $p = 0.01$, respectively). Significant differences were also found between prosthesis P4 and P1 (P4 versus P1, $p = 0.01$) as well as P2 and P1 (P2 versus P1, $p = 0.04$).

Burst activity

To test burst activity, *Escherichia coli* bacteria were used as stimulus for phagocytosis and the simultaneous generation of reactive oxygen radicals. The gelatine-coated polyester prosthesis led to a strong reduction in generation of oxygen radicals and subsequent phagocytosis capacity (P4 versus P1, P2 and P3, $p = 0.00$, respectively). The remaining prostheses modulated PMN reactivity only slightly (P3>P2>P1; fig. 3). Interestingly, plasma pretreatment of the various prosthesis showed no effects.

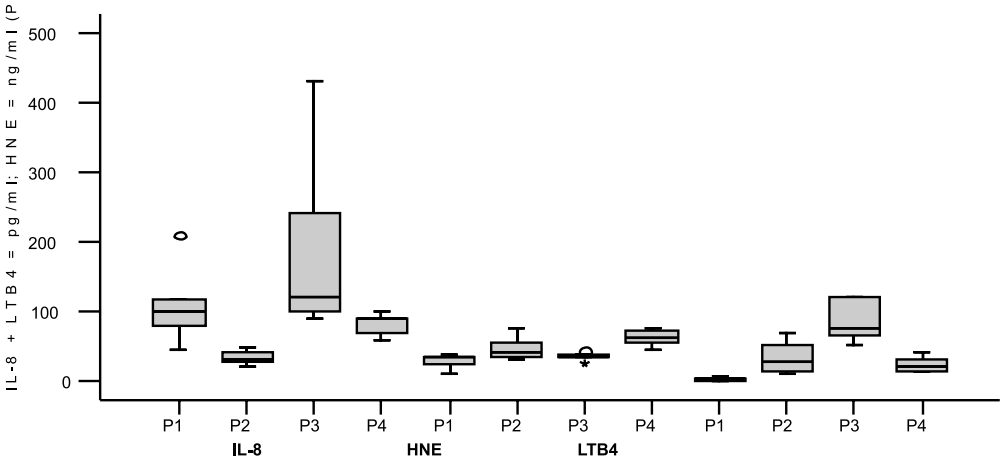

Fig. 2. Inflammatory mediator release from human neutrophils. Changes of IL-8 release (pg/ml), LTB4 generation (pg/ml) and elastase release (ng/ml) from human neutrophils after interaction with uncoated (P1) and silver-coated (P2) vascular grafts are depicted. Data were calculated by substracting the values of generated and released mediators from that of neutrophils exposed to the devices. Experiments were performed in triplicates and data are presented from at least 4 different experiments

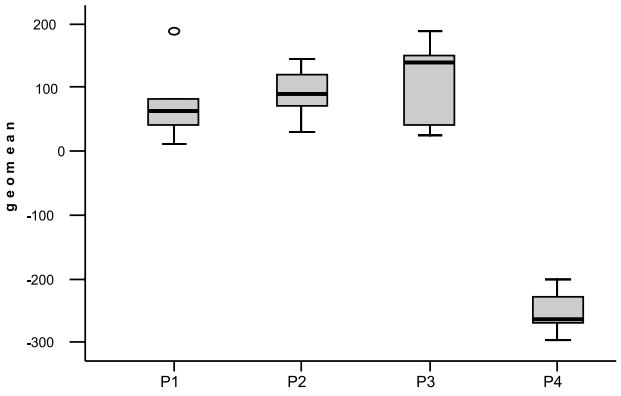

Fig. 3. Oxidative burst activity in human neutrophils. Changes of oxidative burst activity (geomean) in human neutrophils after interaction with uncoated (P1, P3) and gelatine-coated (P2, P4)vascular grafts are presented. Data were calculated by substracting the values of oxidative burst activity in the absence of the devices from that of neutrophils exposed to the devices. Experiments were performed in triplicates and data are shown from at least 8 different experiments

DISCUSSION

The theories on the incorporation of vascular prostheses consider phases of" wound healing" and responses against foreign substances/ biodies (1, 2, 4). Numerous in vitro and in vivo studies have characterized PMN as the effector cells (3, 7, 8). Data on the duration how long granulocytes are present during the incorporation and healing process vary from 1 to 3 weeks in the literature. According to a few authors, even after 6 months PMNs can be still detected (2, 6, 9, 10, 11). Despite the differences in the investigated various cell-to-cell communication within the tissue, PMN may have a great impact onto the incorporation and healing process of vascular grafts but also in case of possible complications (1, 2, 8, 12).

The presented standardized experimental in vitro model can not extensively discussed with regard to available data from the literature. Although, numerous articles on receptors of the cell surface and mediators in PMN have been published, there are only a few reports with a similar experimental approach. For instance, Jakubiec et al. (13) have investigated the up-regulation of Mac-1 (CD11b/ CD18) by analyzing CD18 in isolated PMN of healthy donors after incubation with prosthetic material. Swartbol et al. (14) have brought prosthetic rings and human blood together and determined the receptors of PMN, monocytes and platelets. Gorbet et al. (6) have shown that PMN respond to various artificial material with or with no coating indicated by flow cytometry, which is commonly recommended by numerous study groups for biomaterial tests (6, 13-17).

As shown in the studies mentioned above and as demonstrated in the presented report native polyester was more reactive than PTFE. The reduced response after plasma incubation may be explained by the coupling to PTFE and polyester. Interestingly, there have been no reports published yet on the additional effect of gelatine-coating analyzing a great variety of receptors and mediators. Advantages of a gelatine-coating are comprised by a reduction of bleedings out of stitch holes, prevention of pre-coagulation, possible antibiotic and heparin binding. According to the manufacturer´s instructions, gelatine is hydrolyzed after implantation of the gelatine-coated prosthesis (18). Investigations in plasma-expanding fluid revealed only a marginal PMN receptor up-regulation (CD 11b) under the effect provided by gelatine (19). Wilhelm et al. (20) verified the strongest antigen-restricted immune response induced by gelatine-coated. In addition, Asberg et Videm (21) have demonstrated in systematic studies on biocompatibility that there is a substantially higher cell adhesion rate at the gelatine-coated wall of a cardiopulmonal bypass than at umbilical endothelial cells. In this context, the study groups Utho et al. (22) and Shindo et al. (23) have observed elevated inflammatory parameters by clinical use of gelatine-coated prostheses.

Selectins and integrins as parts of the adhesion cascade at endothelial cells are considered parameters for the getting in touch with the biomaterial (5, 6, 7,8, 13-17, 19, 21). To reflect such cellular processes, CD62L (selectin) and CD11b (CD11b/CD18; αMß$_2$Integrin; Mac-1) were selected. CXCR2 chemokine receptor is important for chemotaxis, cell aktivation (degranulation) and adhesion cascade of the PMN. Since the reexpression of the receptor is distinctly later (within hours) than that of the selective IL-8 receptor CXCR1 (within minutes), reduction of the CXCR2 receptor within the incubation time period was used as comparative parameter (5, 24). Signal transduc-

tion via the fMLP receptor is followed by the release of lysosomal enzymes and toxic oxygen radicals. Initiation of phagocytosis and changes of the cytoskeleton are further, receptor-associated subsequent cellular functions (3, 5, 6, 14, 25-28). All receptor changes can be considered quantifiable active cell processes, which have been well described in the literature. For instance, shedding of the L-selectins is a bioactive process, in which an enzyme (sheddase) alters the molecule at the cell surface. Furthermore, reduced CXCR2 density is caused by an activation of p38 MAPK (mitogen-activated protein kinase) and initiation of receptors. Various authors have shown, that receptor density remains unaffected after selective blockade (5, 21, 24, 25, 29, 30). Both fMLP receptor and CD11b receptor are (membrane-associated) located at the secondary (specific) granula of the PMN. During the activation process of the granulocytes, receptor density is up-regulated. In addition, granula population is released by exocytosis (3, 21, 25, 28, 29, 30). The mediator release can also be considered a proinflammatory response onto the exposure to biomaterial. The regulatory chemokine IL-8 generates extensive effects onto the immune response and its modulation within the interactive process of the various immuncompetent cell types.

The elastase release out of the accumulated granula can also be characterized as bioactive process with deleterious and destructing potential for the tissue. The cellular production of LTB_4 aggravates the PMN-dependent responses including tissue destruction and generation of oxygen radicals (7, 31-34). All PMN receptors and mediators mentioned above have been tested with regard to the biocompatibility of cardiopulmonal bypass-coating, oxgenators and systems for hemodialysis (4, 5, 7, 8, 12, 15, 16, 17, 21, 25, 27). In a previous study, the reporting authors have found an association of the PMN-related receptor alterations with various diseases as well as different types of prosthetic coating (35).

The presented results indicate that there is a more pronounced PMN-related receptor stimulation by gelatine-coated vascular graft material. This coating changes also the PNM response onto the usually almost inert PTFE. However, IL-8 shows a differing course. For the effects of un-coated PTFE, the data from Asberg et Vedem (21) and Welty et al. (36) need to be included, which show that the blockade of the adhesion molecule (CD62L, CD11b/CD18) does not lead to a complete abolishment of the cell adhesions. Due to a compensatory increase of the cytokine expression and production, there are interactions with adhesion receptors of various other cell types in the cellular composite. If there is a gelatine-coating, the cell-dependent responses exceed those onto the un-coated prostheses with a very similar effect comparing the polyester prostheses.

Depending from the incorporation of artificial material in the sift tissue, prosthetic infections become clinically manifest in 60 % of cases within 4 weeks but in 80 % within one year. respectively (1, 2). The close relationship to the single phases of the incorporation and healing process has been demonstrated by several authors in animal studies as well as in explanted prostheses at various time points after implantation. Interestingly, a persisting white blood cell-triggered inflammation or response to a foreign body has been found to be associated with an increased infection rate (37-40). With regard to the pathogenesis, Vaudaux et al. (34) postulated that PMN run out of further potent unspecific infectious defence because of a futile phagocytosis of the foreign material. The presented results show clearly that this process can be altered by different material and/or coating since, e.g., gelatine-coated polyester led to a reduced phagocytosis capacity. Further experiments by Kaplan et al. (41) and Chang et al. (42) have observed a time- and material-dependetn non-apototic cell death. However, the reported time periods were different to the regimen in the presented study. The primary protein layer has also an impact on the biomaterial-tissue interaction, inflammatory and immune response as well as infectious resistance.

Again, experiments revealed that PMN-related responses are altered and bacterial coupling sites are masked or presented (3, 4, 12, 34, 38, 43, 44). In the presented study, plasma coating influenced the PMN-related receptor expression, which was mainly caused by a delamination of gelatine and primary effect of the material. The augmented shedding in native polyester can be explained by an improved contact to the material due to fibrinogen. Several groups have elucidated the specific binding sites (21, 43, 44). Native polyester induced also greater magnitudes fort he mediators IL-8 and

LTB_4 than gelatine-coated polyester did. In contrast, both gelatine-coated polyester and PTFE provoked a more pronounced release of elastase than un-coated material. LTB_4 has been only analysed in the extracorporal circulation (45). It remains to clarify whether i) after decayed rifampicin effect in case of infection, ii) attenuated phagocytosis capacity of the primary unspecific defence cell population, or iii) overstimulation of these cells may affect the incorporation in the intermediate or long-term time course, and, thus, a late or prolonged infection can still occur (3, 5, 7, 12, 21, 23, 24, 27, 29, 31, 34, 38, 40, 41, 42, 46). Modern investigations focussing onto cell-to-cell interactions, cytokine production and release as well as the general histo-/hemocompatibility of the novel generation of vascular prostheses in human cells (35) and experimental models are not available yet. However, presented experimental model and data suggest that the setting is feasible and reliable for the indicated subsequent study subjects and aims.

CONCLUSIONS

Modern vascular surgery is unimaginable without implantation of alloplastic material. The vascular grafts have to fulfil various requirements, e.g., avoidance of blood loss, a low thrombogenicity, adequate bio-/hemocompatibility and mechanic as well as infection resistence. Despite precisely designed surface structures, a proinflammatory response due to a provoked activation of PMN after implantation can not completely avoided.

The presented experimental model and design showed the stimulating impact of gelatine-coating onto these human effector cells indicated by established parameters for an inflammatory/immune response. Within the complex interaction of receptors and mediators, intra- and extracellular response of the effector cells on the alloplastic material is regulated, on which type of coating, plasma and characteristics of the alloplastic material have a substantial impact.

REFERENCES

1. *Vollmar J:* Rekonstruktive Chirurgie der Arterien. 4. Auflage. Stuttgart: Thieme, 1996
2. *Xue L, Greisler HP:* Prosthetic grafts. In: Rutherfort RB (eds) Vascular surgery. 6th edition, vol 1. Philadelphia: *Elsevier Saunders* 2005; 723-40.
3. *Burg ND, Pillinger MH:* The neutrophil: function and regulation in innate and humoral immunity. *Clin Immunol* 2001; 99: 7-17.
4. *Wintermantel E, Mayer J, Ruffieux K* et al.: Biomaterialien – humane Toleranz und Integration. *Chirurg* 1999; 70: 847-57.
5. *Chishti AD, Dark JH, Kesteven P* et al.: Expression of chemokine receptors CXCR1 and CXCR2 during cardiopulmonary bypass. *J Thorac Cardiovasc Surg* 2001; 122: 1162-67.
6. *Gorbet MB, Yeo EL, Sefton MV:* Flow cytometric study of in vitro activation by biomaterials. *Biomed Mater Res* 1999; 44: 289-97.
7. *Munch K, Wolf MF, Gruffaz P* et al.: Use of simple and complex in vitro models for multiparameter characterization of human blood-material/device interactions. *J Biomater Sci Polym Ed* 2000; 11: 1147-63.
8. *Tang L, Ugarova TP, Plow EF, Eaton JW:* Molecular determinants of acute inflammatory responses to biomaterials. *J Clin Invest* 1996; 97: 1329-34.
9. *Charara J, Beaudion G, Fortin C* et al.: In vivo biostability of four types of arterial grafts with impervious walls; their haemodynamic and pathological characteristics. *J Biomed Eng* 1989; 11: 416-28.
10. *Dewanjee MK, Solis E, Mackey ST* et al.: Quantification of deposition of neutrophilic granulocytes on vascular grafts in dogs with ^{111}In-labeled granulocytes. *Mayo Clin Proc* 1985; 60: 173-79.
11. *Pärsson H, Jundzill W, Jonung T* et al.: The adhesion of labelled neutrophils on synthetic vascular grafts. An experimental porcine study. *Eur J Vasc Surg* 1993; 7: 257-62.
12. *Anderson JM, Rodriguez A, Chang DT:* Foreign body reaction to biomaterials. *Semin Immunol* 2008; 20: 86-100.
13. *Jakubiec B, Roy R, Isles MB* et al.: Measurement of CD11/CD18 integrin expression on the polymorphonuclear cell surface after incubation with synthetic vascular prostheses. *ASAIO J* 1994; 40: M616-M618.
14. *Swartbol P, Truedsson L, Parsson H, Norgren L:* Surface adhesion molecule expression on human blood cells induced by vascular graft materials in vitro. *J Biomed Mater Res* 1996; 32: 669-76.
15. *Cenni E, Granchi D, Verri E* et al.: Evaluation of endothelial cell integrins after in vitro contact

with polyethylene terephthalate. *J Mater Sci Mater Med* 2001; 12: 345-49.
16. *Granchi D, Cenni E, Verri E* et al.: Flow-cytometric analysis of leukocyte activation induced by polyethylene-terephthalate with and without pyrolytic carbon coating. *J Biomed Mater Res* 1988; 15: 549-553.
17. *Pu FR, Williams RL, Markkula TK, Hunt JA:* Effects of plasma treated PET and PTFE on expression of adhesion molecules by human endothelial cells in vitro. *Biomaterials* 2002; 23: 2411-28.
18. *Drury JK, Ashton TR, Cunningham JD* et al.: Experimental and clinical experience with a gelatin impregnated Dacron prosthesis. *Ann Vasc Surg* 1987; 1: 542-47.
19. *Engel JM, Welters I, Rupp M* et al.: Influence of colloid fluids on polymorphonuclear granulocyte function in vitro. *Acta Anaesthesiol Scand* 2001; 45: 385-89.
20. *Wilhelm L, Zippel R, von Woedtke T* et al.: Immune response against polyester implants is influenced by the coating substances. *J Biomed Mater Res A* 2007; 83: 104-13.
21. *Asberg AE, Videm V:* Neutrophil dysfunction after biomaterial contact in an in vitro model of cardiopulmonary bypass. *Eur J Cardiothorac Surg* 2006; 30: 744-48.
22. *Utoh J, Goto H, Hirata T* et al.: Postoperative inflammatory reactions to sealed Dracon prostheses: a comparison of Gelseal and Hemashield. *J Cardiovasc Surg* (Torino) 1997; 38: 287-90.
23. *Shindo S, Motohashi S, Katsu M* et al.: Coated prostheses are associated with prolonged inflammation in aortic surgery: a cost analysis. *Artif Organs* 2008; 32: 183-87.
24. *Cummings CJ, Martin TR, Frevert CW* et al.: Expression and function of the chemokine receptors CXCR1 and CXCR2 in sepsis. *J Immunol* 1999; 162: 2341-46.
25. *Graves V, Gabig T, McCarthy L* et al.: Simultaneous mobilization of Mac-1 (CD11b/CD18) and formyl peptide chemoattractant receptors in human neutrophils. Blood 1992; 80 (3): 776-787. Erratum in: *Blood* 1993; 81: 1668.
26. *Heit B, Tavener S, Raharjo E, Kubes P:* An intracellular signaling hierarchy determines direction of migration in opposing chemotactic gradients. *J Cell Biol* 2002; 159: 91-102.
27. *Hughes SF, Cotter MJ, Evans SA* et al.: Role of leucocytes in damage to vascular endothelium during ischaemia-reperfusion injury. *Br J Biomed Sci* 2006; 63: 166-70.
28. *Ley K:* Integration of inflammatory signals by rolling neutrophils. *Immunol Rev* 2002; 186: 8-18.
29. *Bullard DC:* Adhesion molecules in inflammatory diseases: insights from knockout mice. *Immunol Res* 2002; 26: 27-33.
30. *Gao F, Yue TL, Shi DW* et al.: p38 MAPK inhibition reduces myocardial reperfusion injury via inhibition of endothelial adhesion molecule expression and blockade of PMN accumulation. *Cardiovasc Res* 2002; 53: 414-22.
31. *Kasama T, Miwa Y, Isozaki T* et al.: Neutrophil-derived cytokines: potential therapeutic targets in inflammation. *Curr Drug Targets Inflamm Allergy* 2005; 4: 273-79.
32. *Nicholson GC, Tennant RC, Carpenter DC* et al.: A novel flow cytometric assay of human whole blood neutrophil and monocyte CD11b levels: upregulation by chemokines is related to receptor expression, comparison with neutrophil shape change, and effects of a chemokine receptor (CXCR2) antagonist. *Pulm Pharmacol Ther* 2007; 20: 52-59.
33. *Takami M, Terry V, Petruzzelli L:* Signaling pathways involved in IL-8-dependent activation of adhesion through Mac-1. *J Immunol* 2002; 168: 4559-66.
34. *Vaudaux P, Francois P, Lew DP, Waldvogel FA:* Host factors predisposing to influencing therapy of foreign body infections. In: Waldvogel FA, Bisno AL (eds) Infections associated with indwelling medical devices. Washington: ASM Press 2000: 1-26.
35. *Tautenhahn J, Freytag CC, Gebauer T* et al.: Experimentelle Untersuchungen zur Biokompatibilität beschichteter Gefäßprothesen in Abhängigkeit von der Grundkrankheit verschiedener Patientenkollektive. *Zentralbl Chir* 2003; 128: 161-65.
36. *Welty-Wolf KE, Carraway MS, Ghio A* et al.: Proinflammatory cytokines increase in sepsis after antiadhesion molecule therapy. *Shock* 2000; 13: 404-09.
37. *Freytag CC, Tautenhahn J, Koenig W* et al.: Ultrastructural analysis of an infected collagen-coated vascular graft. *Vasa* 2003; 32: 31-35.
38. *Hernandez-Richter T, Wittmann F, Mayr S* et al.: Rifampin and Triclosan but not silver is effective in preventing bacterial infection of vascular Dacron graft material. *Eur J Vasc Endovasc Surg* 2003; 26: 550-57.
39. *Olofsson P, Rabahie GN, Matsumoto K* et al.: Histopathological characteristics of explanted human prosthetic arterial grafts: implications for the prevention and management of graft infection. *Eur J Vasc Endovasc Surg* 1995; 9: 143-51.
40. *Roon AJ, Malone MJ, Moore WS* et al.: Bacteremic infectability: a function of vascular graft material and design. *J Surg Res* 1977; 22: 489-98.
41. *Kaplan SS, Basford RE, Jeong MH, Simmons RL:* Biomaterial-neutrophil interactions: dysregulation of oxidative functions of fresh neutrophils induced by prior neutrophil-biomaterial interaction. *J Biomed Mater Res* 1996; 30: 67-75.
42. *Chang S, Popowich Y, Greco RS, Haimovich B:* Neutrophil survival on biomaterials is determined by surface topography. *J Vasc Surg* 2003; 37: 1082-90.
43. *Falkenback D, Lundberg F, Ribbe E, Ljungh A:* Exposure of plasma proteins on Dacron and ePTFE vascular graft material in a perfusion model. *Eur J Vasc Endovasc Surg* 2000; 19: 468-75.

44. *Keselowsky BG, Bridges AW, Burns KL* et al.: Role of plasma fibronectin in the foreign body response to biomaterials. *Biomaterials* 2007; 28: 3626-31.
45. *Zimmermann AK, Weber N, Lebert H* et al.: Effect of biopassive and bioactive surface coatings on the hemocompatibility of membrane oxygenators. *J Biomed Mater Res B Appl Biomater* 2007; 80: 433-39.
46. *Lappegard KT, Bergseth G, Riesenfeld J* et al.: Role of granulocytes and monocytes in the polyvinyl chloride-induced synthesis of interleukin 8, monocyte chemoattractant protein 1, and leukotriene B4. *J Biomed Mat Res* A 2005; 74: 230-36.

Received: 7.08.2015 r.
Adress corre**spondence:** Lek. med. Joerg Tautenhahn, Klinika Chirurgii Naczyniowej, Szpital Miejski, Magdeburg (Niemcy)
e-mail: joerg.tautenhahn@klinikum-magdeburg.de