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Evaluation of the biocompatibility of a new vascular prosthesis coating by detection of prosthesis-specific antibodies

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Abstract In recent experimental studies, we could demonstrate the occurrence of antibodies against the prosthesis matrix and coating following implantation of polyester-based vascular grafts. Therefore, this study aimed at evaluating the biocompatibility of a new absorbable polymer coating by detection of antibodies against the coating and the polyester matrix. Two polyester vascular prostheses coated either with the polymer (PP-prosthesis) or with gelatine (PG-prosthesis) were functionally implanted into sheep ($n = 22$ per group). Blood was drawn on days 1 (pre-OP) and 7, 14, 28, 56, 84, 140 (post-OP). Homogenates from both prostheses (PP-target or PG-target) or from an uncoated prosthesis (P-target) were used as assay targets in a particle-based immunoassay. The antibody binding against the P-target was significantly higher in the PP-group than in the PG-group on days 7–56, but not on days 84 and 140. Within both groups, no significant differences but a significant correlation between the binding against the P-target and the coated target was found. Therefore, the absorbable polymer did not induce a specific humoral immune response. In conclusion, the overall immunogenicity of the polymer-coated graft was comparable to the gelatine-coated graft. The detection of prosthesis-specific antibodies seems to be useful for in vivo biocompatibility testing.

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1 Introduction

Implantation of biomaterials like textile vascular prostheses is followed by local inflammation consisting of an acute phase as well as a chronically persistent reaction [1]. While this response is an important part of the wound-healing process and therefore a prerequisite for proper implant integration into the surrounding tissue, it may also impair the function of the implant due to biodegradation. Additionally, inflammatory reactions as well as immunological responses against components of the implant might also be responsible for clinical complications following implantation [2]. The properties of a prosthesis, and especially the features of its surface at the contact interface to the surrounding biological tissue, are therefore of central importance for its biocompatibility and the long-term outcome after implantation. Several approaches have been studied to improve the biocompatibility of vascular protheses, for example different surface textures and porosity, as well as coating of the surface with proteins such as collagen, gelatine or albumin, or with other materials. Additionally, the impregnation of the graft surface also

serves as a sealent, thereby eliminating the time-consuming process of preclotting with patients blood prior to implantation [3].

Within the context of implant-related inflammation, phagocytes have been described as the primary cell population [4]. Additionally, newer studies implicate other cells as well, for example mast cells [5] as well as lymphocytes and particularly T-lymphocytes [6]. However, little is known regarding specific humoral and cellular immune responses against synthetic biomaterials [7]. Since phagocytes process foreign structures and present antigens to immunocompetent cells, they are a major link between inflammation and the immunological response. In the context of biomaterials, phagocytic cells might process small particles which are released from the implant by biodegradation or physical abrasion. Consequently, they might trigger reactions which can subsequently lead to humoral markers such as specific antibodies (Abs) against implant components. So far, studies on the occurrence of such Abs against the coating [8, 9] or the polymer material [10, 11] of different implants have shown conflicting results.

Using a new immunoassay format based on prosthesis segments or on particles from prosthesis homogenates, we were able to demonstrate the occurrence of material-specific Abs following implantation of polyester vascular grafts [12–14] in experimental studies using different animal models and implantation sites. Additionally, we found Abs against albumin [14] and collagen [15] used for graft coating/impregnation. Moreover, our results demonstrated a distinct influence of the prosthesis coating on the Ab response against the prosthesis matrix [14]. Moreover, we recently found a possible relationship between the development of prosthesis-specific Abs and the local tissue reactions at the implantation site [16].

The detection of such Abs could therefore be a useful method for the examination of the biocompatibility of new or improved biomaterials. Therefore, the aim of the current study was to use the detection of Abs against uncoated and coated vascular graft material to evaluate the biocompatibility of a new absorbable polymer in a sheep model. For comparison, an established prosthesis which is approved for clinical use and made from the same polyester material with a gelatine impregnation was used as control.

2 Materials and methods

2.1 Prostheses

Two different vascular prostheses (diameter 8 mm) which were made from a knitted polyester material were used for the implantation experiments. The first one, designated "PG-prosthesis", was a commercially available prosthesis which is approved for clinical use and which served as control in this study. It was pre-coated by the manufacturer with gelatine using a proprietary coating procedure. The second one, designated "PP-prosthesis", was coated with a newly developed absorbable polymer, consisting of lactide, caprolactone, trimethlyene carbonate and glycolide, instead of gelatine.

For the immunoassay experiments, an additional third prosthesis was used. This one, designated "P-prosthesis", consisted of the same polyester matrix but did not receive an additional coating.

2.2 Study design

Forty four adult sheep were randomly divided into two groups. All animals of each group received one of the two coated prostheses functionally implanted as carotid artery bypass. The experimental groups were designated according to their prosthesis type as "PG-group" and "PP-group", respectively. The anaesthesia and implantation procedures were performed according to standard surgical techniques (details available from the authors). Blood was drawn on day 1 (pre-OP) and on days 7, 14, 28, 56, 84, with an additional blood sampling on day 140 for half of each group ($n = 11$). All aspects of the animal experiments were conducted in accordance with the German animal protection law, with the principles of care for animals in laboratories (drawn up by the National Society for Medical Research) and with the Guidelines for Keeping and Using Laboratory Animals (NIH Publication No.80-23, revised 1985).

2.3 Immunoassays for antibody detection

Abs against the prostheses were detected by enzyme immunoassays using μm-sized prosthesis homogenate particles as assay targets. The preparation of these particles and the assay procedure are described in detail elsewhere [12, 14]. Briefly, 1.0 g of each prosthesis material was dispersed in 100 ml PBS/0.09% NaN_3 using an Ultra-Turrax. The resulting particle suspensions were designated "PP-target" for the polymer-coated prosthesis material (total particle count: $n = 77{,}000$ per μL; size <1 μm: $n = 47{,}000$ per μL), "PG-target" for the gelatine-coated prosthesis material (total particle count: $n = 82{,}500$ per μL; size <1 μm: $n = 48{,}750$ per μL), and "P-target" for the uncoated prosthesis material (total particle count: $n = 52{,}250$ per μL; size <1 μm: $n = 42{,}500$ per μL), respectively.

The detection of Abs against the prostheses was performed in 96-well microtiter plates. As a blocking step,

50 μL particle suspension at a 1/4 dilution in PBS/ 2% BSA (Sigma, Deisenhofen, Germany) per well, corresponding to 0.6×10^6 particles <1 μm for the PP- and PG-targets and about 0.5×10^6 particles <1 μm for the P-target, were pipetted into V-well plates (Greiner, Frickenhausen, Germany) and incubated for 90 min at 25 °C. After centrifugation at 1,100*g* and blocking buffer removal, the particles were incubated with 50 μL sheep serum samples at dilutions of 1/50 and 1/100 in SuperBlock (Pierce, Bonn, Germany) overnight at 25 °C while shaking. After three washing steps with PBS/1% BSA/0.05% Tween 20, 50 μL of a horseradish peroxidase labelled rabbit anti-sheep IgG antibody (Fcγ–chain specific, Nordic Immunological Laboratories, Tilburg, The Netherlands) diluted 1:1,000 in SuperBlock were added to each well and incubated for 60 min at 25 °C while shaking. After washing, the particle pellets were re-suspended in 125 μL per well of TMB chromogenic substrate solution (Sigma, Deisenhofen, Germany), and 100 μL of the suspension were immediately transferred into a new flat-bottom plate. After 30 min incubation in the dark, 50 μL of 2 mol/l sulphuric acid were added to stop the substrate reaction. The optical density (wavelength 490/630 nm) was measured using a MRX Revelation microtiter plate reader (Dynatech laboratories, Inc., Chantilly, VA, USA).

Serum samples which gave negative respectively high positive readings in preliminary experiments served as negative and positive control on each plate throughout the study.

2.4 Data processing and statistical analysis

To take individual as well as day-to-day variations into account, the raw OD values for each individual animal were adjusted and normalized using the plate-specific positive and negative control values according to the following formula: $OD_{\text{normalized, adjusted}} = OD_{\text{Raw value}}/OD_{\text{Positive control}} - OD_{\text{Negative control}}/OD_{\text{Positive control}}$. From the resulting data for all experimental days, the normalized and adjusted individual pre-OP value (day 1) was deducted as a base line (also resulting in negative values). For all animals, the data represent the average of the normalized and adjusted values from two immunoassay determinations performed on different days, determined in replicates on each day.

The median Ab binding on different experimental days between both groups or between two targets in the same group was compared with the Mann–Whitney-test. The correlation between the binding against the uncoated and the coated targets in both groups was analyzed using the non-parametric Spearman's test. The statistical analysis was performed with GraphPad Prism version 4.02 (GraphPad Software, Inc., San Diego, CA, USA).

3 Results

3.1 Antibody binding in the PP-group (Table 1)

In the PP-group, the Ab binding against the P-target did not differ significantly in comparison to the PP-target on any experimental day ($p = 0.7012$ over all experimental days). A significant correlation was found between the Ab binding against both targets (Fig. 1; $p < 0.0001$, $r_S = 0.6807$). In accordance with this finding, the responses against the P-target and the PP-target were found to be similar regarding time course and extent in most individual animals of this group. The highest binding against both targets was found on day 14 (P-target: $p = 0.0099$; PP-target: $p = 0.0289$, both compared vs. day 7) and day 28 (P-target: $p = 0.0118$ compared versus day 7). After that, a pronounced decline was observed until the end of the study period, with significantly lower values for the P-target on day 84 ($p = 0.0383$) and day 140 ($p = 0.0022$) compared to day 14.

3.2 Antibody binding in the PG-group (Table 1)

In the PG-group, there was no significant difference between the Ab binding against the P-target and the PG-target on any experimental day ($p = 0.0863$ over all experimental days). The Ab binding against both targets was significantly correlated (Fig. 2; $p < 0.0001$, $r_S = 0.5362$). The maximum binding against the PG-target in this group occurred on day 14 and day 28 ($p = 0.0026$ and $p = 0.034$, respectively, compared versus day 7). While it declined after the maximum, it was not significantly different on days 84 and 140 compared to day 14 or 28. The Ab binding against the P-target in this group was elevated on day 14 in comparison to day 7 and remained relatively constant afterwards until the end of the study. It was not significantly different from day 7 on any experimental day. An additional observation in the PG-group was that some but not all animals demonstrated a pronounced early peak in their response against the PG-target which was markedly different from the time course of the response against the P-target (data not shown).

3.3 Comparison between both groups (Table 1)

In comparison between both groups, the Ab binding against the P-target was significantly higher in the PP-group than in the PG-group (0.2210 vs. 0.1060 over all days; $p < 0.0001$). Significant intra-day differences were found on day 7, 14, 28 and 56. Comparing the Ab binding against the coated prostheses in both groups, the binding against

Table 1 Antibody binding in the PP-group and the PG-group against the P-target (uncoated prosthesis matrix) and PP-respectively PG-target (coated prosthesis material). Data represent the adjusted and normalized OD values; see details in 'Data processing and statistical analysis'

	All days	Day 7	Day 14	Day 28	Day 56	Day 84	Day 140
PP-group							
P-target Median (IQR)	0.221 (0.058–0.387)	0.103 (0.029–0.241)	0.325 (0.135–0.479)	0.292 (0.199–0.460)	0.243 (0.115–0.349)	0.194 (0.031–0.345)	0.045 (−0.020–0.179)
PP-target Median (IQR)	0.200 (0.020–0.408)	0.148 (−0.014–0.313)	0.287 (0.079–0.560)	0.275 (0.070–0.505)	0.2140 (0.096–0.365)	0.173 (−0.017–0.326)	0.042 (−0.083–0.460)
P- vs. PP-target (*p*-value)	0.7012	0.9690	0.8120	0.6925	0.4208	0.9246	1.0000
PG-group							
P-target Median (IQR)	0.106 (0.021–0.209)	0.037 (−0.119–0.099)	0.113 (0.021–0.246)	0.130 (0.039–0.339)	0.125 (0.019–0.246)	0.107 (−0.030–0.162)	0.105 (0.010–0.281)
PG-target Median (IQR)	0.037 (−0.153–0.264)	−0.082 (−0.268–0.048)	0.187 (−0.010–0.408)	0.252 (−0.002–0.517)	0.005 (−0.157–0.213)	0.005 (−0.160–0.202)	0.023 (−0.225–0.349)
P- vs. PG-target (*p*-value)	0.0863	0.0811	0.6073	0.6304	0.1102	0.2801	0.5787
PP- vs. PG-group							
P- vs. P-target (*p*-value)	<0.0001	0.0367	0.0093	0.0138	0.0469	0.1136	0.5149
PP- vs. PG-target (*p*-value)	<0.0001	0.0012	0.2230	0.4675	0.0287	0.0722	0.3418

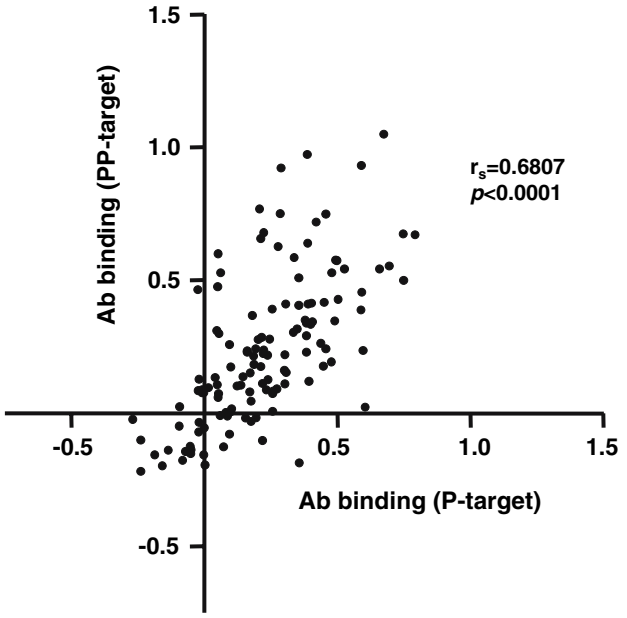

Fig. 1 Median antibody binding against the P-target versus PP-target of all individual animals on all experimental days in the PP-group. The Ab binding to both targets reveals a significant correlation (Spearman's correlation coefficient $r_S = 0.6807$; $p < 0.0001$)

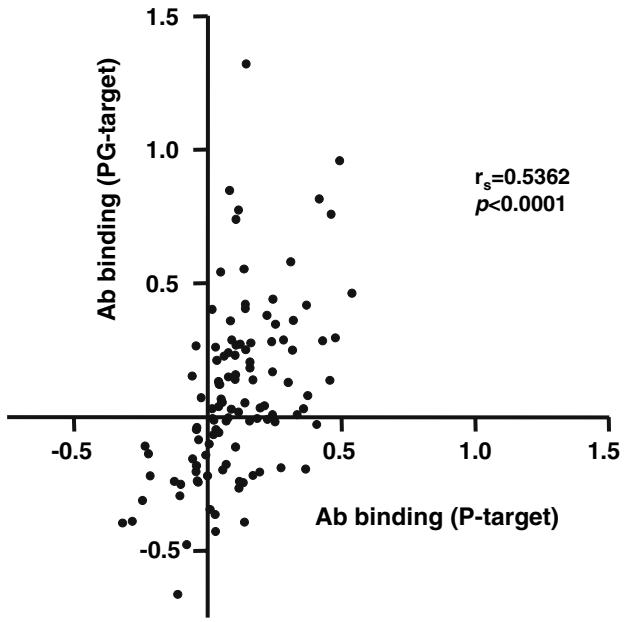

Fig. 2 Median antibody binding against the P-target versus PG-target of all individual animals on all experimental days in the PG-group. The Ab binding to both targets reveals a significant correlation (Spearman's correlation coefficient $r_S = 0.5362$; $p < 0.0001$)

the PP-target in the PP-group was significantly higher than against the PG-target in the PG-group (0.2005 vs. 0.0370 over all days; $p < 0.0001$). Significant intra-day differences between both groups were observed on days 7 and 56. In contrast, no significant differences between both groups were observed on days 84 and 140 for both targets.

4 Discussion

The successful clinical outcome of graft implantation depends on the long-term functionality of the implant as well as an appropriate biocompatibility. Both of these aspects are largely influenced by inflammatory and immunological reactions of the body [1, 4–6]. Since most biomaterials are assumed to be non-immunogenic based on their properties and empirical experiences, little is known about specific immune reactions against implanted synthetic biomaterials [7]. This is certainly true for polyester materials which are, among other applications, also used for vascular prostheses. Earlier studies on the development of specific Abs against implanted biomaterials gave conflicting results [8–11]. In a number of recent studies, we were able to consistently demonstrate the occurrence of Abs against the polymer matrix as well as the coating of vascular prostheses using different animal models and implantation sites [12–16]. Therefore, the aim of the current study was to evaluate the biocompatibility of a new absorbable polymer for coating of a polyester-based vascular graft (PP-prosthesis) in comparison with an established graft with a gelatine impregnation (PG-prosthesis), using the detection of Abs against the polyester matrix as well as the coated prostheses after functional implantation in sheep.

The results demonstrate a specific Ab binding in both groups against the uncoated prosthesis matrix (P-target) as well as against the respective coated prosthesis (PP-target and PG-target, respectively). In the PP-group, no differences but a significant correlation between the bindings against both targets were found. The maximum Ab response occurred on days 14 and 28, afterwards a pronounced decline was observed. Such an early increase and later decline of the humoral immune response is comparable to previous results obtained after functional implantation of collagen-coated vascular prostheses in pigs [12]. Our findings indicate that the new absorbable polymer coating does not induce a specific humoral immune response. In principle, this is an advantageous observation in favour of the new polymer coating with a possible relevance for clinical applications. In the PG-group, there were also no significant differences between the non-coated and gelatine-coated targets. Similar to the PP-group, the binding to both targets demonstrated a significant correlation. The highest Ab binding in this group, which was significantly elevated compared to day 7 only for the PG-target, was observed on day 14 and 28. In contrast, while the Ab binding against the P-target in this group was elevated from day 14 and remained relatively constant until day 140, it was not significantly different from day 7 on any experimental day. These results are consistent with the observation that a number of individual animals in the PG-group demonstrated a high and early peak in their response against the PG-target but not to the P-target. This indicates a specific Ab response against the gelatine coating in these animals as also observed recently in a pig model [15].

Comparing both groups, the Ab response against the graft matrix polyester was significantly higher (2- to 3-fold) in the PP-group than in the PG-group from days 7–56. Similarly, the response against the PP-target in the PP-group was also significantly higher than the response against the PG-target in the PG-group. Therefore, while the newly developed absorbable polymer does not induce a specific humoral immune response, the polymer-coated prosthesis demonstrated a higher immunogenicity of the polyester matrix compared to the gelatine-coated prosthesis in the early phase. However, later in the investigational period on days 84 and 140, the binding against the polyester matrix was not significantly different between both groups. This seems to indicate that the long-term immunogenicity of the polymer-coated prosthesis is comparable to the established gelatine-coated prosthesis.

Following our previous experiments in rats [13, 14] and pigs [12, 15, 16], the sheep model used in the current study is the second experimental model based on functional implantation of vascular prostheses in a large animal species. The results support our previous findings that polyester materials which are used as vascular graft matrix induce a specific Ab response. Furthermore, these data demonstrate that different coating materials exhibit a variable influence on the Ab response against the graft matrix. This is the first such finding after functional implantation in a large animal species and confirms earlier results which were performed in a rodent model using intramuscular implantation in rats [14]. Although the differences between experimental animal models and the human body regarding the immune system and the blood circulation have to be kept in mind for judging these results, functional implantation in large animals can be assumed to parallel the clinical situation in many important aspects.

The antigenic structures which are responsible for the development of Abs against polyester remain unknown. However, it can be assumed that microparticles and other leachables caused by biodegradation and physical abrasion processes play an important role in this process. While the possible clinical relevance of prosthesis-specific Abs remains to be seen, the occurrence of these Abs is an indicator of the immunogenicity of a biomaterial. Therefore, the detection of specific Abs seems to be an additional useful method in the evaluation of the biocompatibility of new implant materials.

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