ORIGINAL RESEARCH

Comparison of Long-Term Biocompatibility of PVDF and PP Meshes

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ABSTRACT

Background: Abdominal hernia repair is the most frequently performed operation in surgery. Mesh repair in hernia surgery has become an integral component. Although meshes made of PVDF are already in clinical use, so far no data of long-term biocompatibility are available. Methods: In this study a PVDF mesh was compared to a polypropylene mesh with regard to its long-term biocompatibility. A total of 28 rats were randomized to two groups. Mesh material was implanted subcutaneously; animals were euthanized seven days and six months postoperatively. The quantity of inflammatory tissue response was characterized by measuring the diameter of the foreign body granuloma. Furthermore quality of cellular immune response (T-lymphocytes, macrophages, and neutrophils), and inflammation (COX-2) was analyzed by immunohistochemistry. Furthermore the collagen type I/III ratio was determined. Results: Macrophages, T-lymphocytes, neutrophils, and COX-2 declined significantly up to six months postoperatively in comparison to day 7 for both PVDF and PP meshes, and in both groups the collagen ratio increased significantly in the course of time. PVDF meshes showed a foreign body granuloma size significantly reduced compared to PP (7 days: 20 ± 2 µm vs. 27 ± 2 µm; 6 months 15 ± 2 µm vs. 22 ± 3 µm; p < .001). However no significant differences were found analyzing cellular response six months postoperatively. Conclusions: Our current data suggest that even in the long-term course after six months and despite a higher effective surface of the PVDF samples it showed a smaller foreign body granuloma than with PP whereas the cellular response was similar.

Keywords: abdominal wall hernia, mesh repair, PVDF, Polypropylene, foreign body reaction, biocompatibility

INTRODUCTION

The implantation of nonresorbable mesh prosthesis has become an integral component of surgery in the case of abdominal wall hernia [1]. Although the use of mesh material led to a significant reduction of recurrence rates, the implantation of alloplastic mesh material sometimes is associated with serious mesh infection [2–4], chronic pain [5], or adhesion formation with erosion of adjacent organs and consecutive fistula formation [6, 7]. Apart from predetermined characteristics like strength and elasticity it is the specific tissue response to the mesh material that defines the suitability of a polymer [8]. Most of the meshes used for hernia repair are constructed of polypropylene (PP), a polymer known for its initial inflammatory and consecutive fibrotic reaction [9]. Efforts were made to improve biocompatibility by introducing low-weight large porous and elastic samples [10]. In 2002 polyvinylidenefluoride (PVDF) was introduced as a new polymer for surgical meshes [8]. PVDF is more resistant to hydrolysis and degradation in comparison to polyester and polypropylene [11]. Whereas the local tissue response to PVDF is quite moderate within the first weeks after implantation [8], little is known about the long-term cellular reactions after more than three months. The aim of this study was to determine the long-term biocompatibility of PVDF meshes in comparison to PP meshes in a rodent model supported by immunohistochemical examination up to six months postoperatively. As markers for inflammatory activity we analyzed the
expression of cyclooxygenase-2 (COX-2) and the number of macrophages (CD68), T-lymphocytes (CD3), and neutrophile granulocytes (myeloperoxidase) at the interface.

**MATERIALS AND METHODS**

A total of 28 male Wistar rats with a mean bodyweight of 400 g were randomly divided into two groups consisting of a PVDF group (n = 14) and a PP group (n = 14). All animals were kept under standardized conditions: temperature between 22°C and 24°C; relative humidity 50–60%; 12 hr of light following 12 hr of darkness. The animals had free access to food and water. Food was withdrawn 12 hr before and after surgery. All operations were carried out under general anesthesia and aseptic and sterile surgical conditions.

**Mesh Materials**

Mesh materials used for this study were a PP mesh (FEG Textiletechnik, Aachen Germany) with an effective surface of 1.1 m²/m², pore size of >2.0 mm and a filament diameter of 140 µm and a PVDF mesh (FEG Textiletechnik, Aachen Germany) with an effective surface of 2.0 m²/m², pore size of >1.0 mm, and a filament diameter of 160 µm.

**Surgical Procedure**

After induction by isoflurane, general anesthesia was achieved with a subcutaneous mixture of 0.3 mg/kg medetomidine and ketamine hydrochloride 100 mg/kg. The rats were weighed, and their skin was shaved and disinfected with a polyvidone-iodine solution. The animals were fixed in a supine position. A 2 cm long paramedian skin incision was performed in the left and right lower abdomen. By blunt dissection of the subcutaneous tissue, pocket-like cavities were created with a size of 1 × 2 cm. A 1 × 1 cm mesh was implanted subcutaneously. In each animal two of the same polymeric mesh materials were implanted bilateral of the abdominal midline respectively. Skin closure was performed by continuous suture obtained with 3/0 polypropylene (Prolene®; Ethicon Inc., Somerville, NJ, USA). On the day of surgery all animals received buprenorphin (0.05–1 mg/kg) as postoperative analgesia subcutaneously. Seven days and six months after mesh implantation animals in each group were euthanized by isoflurane asphyxiation and decapitation. Tissue specimens for histological and immunohistochemical observations were immediately fixed in 10% formaldehyde.

**Histological Assessment and Immunohistochemical Analysis**

All histological and immunohistochemical investigations were performed on paraffin embedded 3 µm sections using peroxidase-conjugated, affinity-isolated immunoglobulins. All sections were routinely stained with haematoxylin and eosin (H&E) and processed at the same time to reduce internal staining variations. Briefly, immunohistochemistry was done subjected to the avidin-biotin-complex method (ABC) and diaminobenzidine as a chromogen in accordance with the instructions of the manufacturer.

The amount of inflammatory and connective tissue formation was analyzed semiquantitatively by measuring the complete diameter of the foreign body granuloma representing the inflammatory infiltrate and the fibrotic tissue reaction. After capturing four granulomas per sample with a digital camera (Olympus C-3030, Olympus, Hamburg, Germany) separate measurements of four quadrants were performed with the help of a digital image analyzing software (Image-Pro Plus, Media Cybernetics, Silver Spring, MD, USA). Histological investigations were performed by two blinded and independent observers.

Macrophages (CD68) were identified by a 1:50 mouse monoclonal antibody from Dako (Glostrup, Denmark), pretreatment of the fixed specimen with microwave three times, citrate-buffer pH 6, and as secondary antibody rabbit antimouse 1:300 Dako (Glostrup, Denmark). T-lymphocytes (CD3) were identified by a mouse monoclonal antibody, pretreatment with Trypsin, dilution 1:25 (Santa Cruz, CA, USA), and as secondary antibody: rabbit antimouse, dilution 1:300 (Dako, Glostrup, Denmark). Myeloperoxidase (MPO) detection was carried out by antibody rabbit polyclonal antibody, pretreatment with microwave with citrate-buffer pH 6, dilution 1:50 (Abcam, UK), and as secondary antibody goat antirabbit, dilution 1:500 (Dako, Glostrup, Denmark). COX-2 detection was carried out by a 1:100 rabbit monoclonal antibody from DCS (Hamburg, Germany), pretreatment microwave three times, citrat-buffer pH 6, and as secondary antibody goat antirabbit 1:300 Dako (Glostrup, Denmark). All sections were examined by standard light microscopy (Olympus BX51, Olympus, Hamburg, Germany). The percentage of positively stained cells was assessed within the interface of meshes to host tissue (area 100 µm × 100 µm, 400-fold magnification) and captured by a digital camera respectively (Olympus C-3030, Olympus, Hamburg, Germany). Analysis was performed using a digital image analyzing software (Image-Pro Plus, Media Cybernetics, Silver Spring, MD, USA). Ten measurements were performed in each animal, for each mesh and each implantation period. The expression of immunohistochemical parameters was classified by two independent, blinded observers.

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Collagen Type I/III Ratio by Cross-Polarization Microscopy

For cross-polarization microscopy (CPM), 5 µm sections were stained for 1 hr in picrosirius solution (.1% solution of Sirius Red F3BA in saturated aqueous picric acid, pH 2) using the published technique reported by Junqueira [12]. The sections were washed for 2 min in .01 N HCl, dehydrated, cleared, and mounted in synthetic resin. To analyze collagen type I/III ratios, tissue samples were evaluated using CPM. Thicker collagen type I fibers were stained in red-orange shades, whereas thinner collagen type III appeared as pale-green shades. For each sample, 10 regions within the interface (400×, area 100×100 µm) were captured by a digital camera (Olympus C-3030, Hamburg, Germany). Collagen I/III ratios were obtained by analysis of the area of collagen type I and III using digital image analyzing software (Image-Pro Plus, Media Cybernetics, Silver Spring, MD, USA). Results were expressed as a ratio of the area of collagen type I to III.

Statistical Analysis

Statistical analysis has been carried out using the Statistical Package for Social Sciences software (SPSS®, Vers. 17.0, Chicago, IL, USA). Differences between the study groups were analyzed by a Mann–Whitney U-test. p-values < .05 were considered to be significant. All data were represented as mean ± standard deviation.

RESULTS

On the day of surgery, all animals were returned to normal activity. None of the animals died during the postoperative observation periods. None of the rats exhibited signs of postoperative wound infection at the time of explantation. In all animals the reactive cellular infiltrates surrounding the implanted suture materials were of comparable shape. Exemplary electron microscopy of explanted samples revealed the signs of surface cracking of the PP samples which were not detectable on the PVDF samples [11] [Figure 1].

Size of Granuloma

Analyzing the size of the granuloma we found significantly smaller granuloma sizes in the PVDF group than in the PP group after seven days and after six months (Table 1). In both groups, PVDF and PP, the size of the granuloma was significantly thinner after six months than after seven days (Table 2).

Immunohistochemical Observations

Analyzing CD68 we found most of CD68-positive macrophages within the inflammatory infiltrate close to the fiber. After seven days the expression of CD68 was significantly higher in the PVDF group compared with PP (21.2 ± 3.8 vs. 17.3 ± 4.8; p = 0.037). No significant differences could be found after six months. Over time a significant reduction of CD68 expression could be observed in both groups [Table 2, Figure 2].

CD3-positive T-lymphocytes were seen only occasionally at the mesh to host interface. However, after seven days the low expression of CD3 was similar in the PVDF and PP groups. After six months no T-lymphocytes could be detected by immunohistochemistry at all [Table 2, Figure 3].

Although only a few cells, mainly at the outer part of the perifilamentary infiltrate, expressed COX-2, there was a significant reduction of COX-2 expression over time in both study groups (Table 2). A significantly higher expression of COX-2 could be observed only in the PP group after seven days [Figure 4].
MPO expression as marker of neutrophiles was rather low in all study groups. However, we observed a significant reduction of MPO expression after six months in both study groups (Table 2) without any significant differences between the two mesh materials [Table 1, Figure 5].

**Collagen Type I/III Ratio**

The collagen type I/III ratio reflecting the maturity of the scar was similar for the two meshes without showing any significant differences (Table 1); however, after six months the collagen type I/III ratio was significantly elevated for PVDF and PP compared to the that on the seventh postoperative day (PVDF levels were 8.0 ± 0.9 vs. 4.8 ± 0.5 and PP levels were 8.2 ± 1.4 vs. 4.3 ± 0.6; p < .001; Table 2).

**DISCUSSION**

The aim of hernia surgery is permanent reinforcement of the abdominal wall, to avoid recurrence. As such the demand on alloplastic material includes the absence of any polymer degradation that prevents any functional impairment due to excessive inflammation and fibrosis. Better biocompatibility has already been achieved by diversifying the structure of the implanted mesh [10]. Recent studies tried to optimize the polymer surface to reach better incorporation of the mesh implant [13].

The aim of this study was to determine the quality of tissue integration of a PVDF mesh in comparison to a PP mesh in a rat model after up to six months, with a focus on the long-term stability and biocompatibility of both meshes. The foreign body reaction of PVDF and PP appear quite similar to those seen in humans [14, 15]. In accordance with Conze et al. PVDF showed a reduced inflammatory and fibrotic reaction compared to PP at both intervals [9] indicated by a smaller size of the foreign body granuloma even after six months.

Foreign body reactions to alloplastic mesh material is induced by inflammatory cells like macrophages [16, 17] and T-lymphocytes [18, 19]. Eventually, the polymer fibres are surrounded by a cellular infiltrate and a fibrotic capsule of fibroblasts and extended deposits of collagen [20, 21]. CD68-positive macrophages reach their maximum level on second day after injury and slowly decline afterward [22]. We found higher expression of CD68 after seven days. After six months CD68 expression is barely present in both groups indicating that the inflammatory process has diminished.

Since the cell surface marker CD3 indicates an activation of T-lymphocytes we found low expression of CD3 in all study groups showing the adequate integration of the mesh material in the tissue. Although their specific role in dealing with foreign bodies is ill-defined, T-lymphocytes are well known to be important for triggering wound healing.

**TABLE 1** Comparison of the granuloma size, collagen quotient, and immunohistochemical markers depending on used mesh. Data shown are mean ± SD

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<th>PVDF (n = 14)</th>
<th>PP (n = 14)</th>
<th>p-value</th>
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<tr>
<td>Granuloma size in μm</td>
<td>20 ± 2</td>
<td>27 ± 2</td>
<td>.001</td>
</tr>
<tr>
<td>CD68% positive cells</td>
<td>21.2 ± 3.8</td>
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<td>.037</td>
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<tr>
<td>CD3% positive cells</td>
<td>8.8 ± 6</td>
<td>.5 ± .3</td>
<td>.206</td>
</tr>
<tr>
<td>COX2% positive cells</td>
<td>0.5 ± .7</td>
<td>1.6 ± 1.3</td>
<td>.001</td>
</tr>
<tr>
<td>MPO% positive cells</td>
<td>4.4 ± 3.8</td>
<td>2.5 ± .7</td>
<td>.218</td>
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<tr>
<td>Collagen I/III quotient</td>
<td>4.8 ± .5</td>
<td>4.3 ± .6</td>
<td>.051</td>
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**TABLE 2** Comparison of granuloma size, collagen quotient and immunohistochemical markers depending on time. Data shown are mean ± SD

<table>
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FIGURE 2  Expression of CD68 within the inflammatory infiltrate close to the PVDF fiber (A) after seven days and six months (B). Expression of CD68 within the inflammatory infiltrate close to the PP fiber (C) after seven days and six months (D) (magnification 400×).

FIGURE 3  Expression of CD3 in the granuloma of PVDF (A) and PP (B) after seven days (magnification 400×).

FIGURE 4  Expression of COX-2 in the granuloma of PVDF (A) and PP (B) after seven days (magnification 400×).
In the present study, myeloperoxidase activity was used to determine the recruitment of neutrophils into injury site [23]. Myeloperoxidase activity showed a weak activation after seven days independent of the mesh material. After six months the expression of MPO tends toward zero in both groups indicating that neutrophiles—although an essential part of the innate immune system—are not major players in the foreign body reaction. As they are typically attracted by bacterial infections, the absence after six months indicated a lack of infection.

COX-2 is a regulatory factor in the biosynthesis of prostanoids, such as prostaglandins, prostacycline, and thromboxanes [24–26]. COX-2 expression is induced by a variety of agents, including growth factors and cytokines [27–30]. In animal models COX-2 expression increases rapidly in response to inflammatory stimuli and tissue damage [31–35]. However, in vivo models of wound healing the expression of COX-2 is normalized within weeks after injury [36]. We found elevated expression of COX-2 in the PP group suggesting that the decreased granuloma size noted in the PVD group is favorable. Though COX-2 has been described to be present in normal wound healing, we did not find COX-2 expression in our samples after six months indicating that COX-2 is not involved in a low level chronic foreign body reaction.

Analyzing the collagen types I and III we found similar ratios in comparison of PVDF and PP. In normal skin of humans, type I and III collagens exist in a ratio of approximately 10:1 [37]. An increased amount of premature collagen type III is present in the early phase of wound healing and in the presence of inflammatory cells. Later on it is replaced by highly cross-linked and stable collagen type I [38]. A lowered collagen type I/III ratio is claimed to be a predictor of delayed wound healing [39-41] or of an immature scar due to a persistent chronic inflammatory process as seen in the presence of polymeric mesh materials [42]. In our series the collagen type I/III ratio was significantly elevated reflecting the maturing of the scar tissue. This demonstrated an adequate tissue integration of both PVD and PP. In synopsis of the results of the collagen type I/III ratio with the basal level of perifilamentary inflammation six months postoperatively an almost completed wound healing has to be adopted.

Although there are many experimental studies dealing with the analysis of tissue reaction for PP and PVDF meshes, so far there are no long-term results of PVDF meshes available. Our study clearly shows an excellent biocompatibility of the PVDF meshes not only in the short run. To conclude, PVDF shows low inflammation parameters and mature scar formation after six months. The present data clearly show that PVDF is a possible alternative to PP despite an increased effective surface area of the PVDF samples. In particular, the excellent biocompatibility and the manageable tissue reaction at the interface favor the construction of hernia meshes of PVDF to reduce mesh-related side effects. However, rodent models have their natural limitations,
long-term biocompatibility of the PVDF meshes. Clinical studies have to be performed to confirm the underlying human disease or comorbidity. Therefore, the animals cannot reflect any situation. In particular, the animals cannot reflect any and results cannot be translated directly to the human situation. In particular, the animals cannot reflect any underlying human disease or comorbidity. Therefore, clinical studies have to be performed to confirm the long-term biocompatibility of the PVDF meshes.

Declaration of Interest: The authors report no conflicts of interest.

REFERENCES


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