Retrieval study at 623 human mesh explants made of polypropylene – impact of mesh class and indication for mesh removal on tissue reaction

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Received December 3, 2012; revised March 4, 2013; accepted March 27, 2013
Published online in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/jbmb.32958

Abstract: Textile meshes frequently are implanted in the abdominal wall to reinforce a hernia repair. However, revisions for mesh associated complications confirm that these devices are not completely free of risk. Explanted devices offer an opportunity to define the impact of mesh structure on tissue response. This retrieval study analyses the tissue reaction to 623 polypropylene mesh samples (170 class 1 with large pores, and 453 class 2 with small pores) explanted for pain, infection, or recurrence. Histopathological assessment included morphometry of inflammatory infiltrate (IF) and connective tissue (CT), and of collagen 1/3 ratio. Half of the meshes were removed after more than 23 month. Despite large inter-individual differences removal for infection showed more IF than for pain or recurrence with significant correlation of IF with CT. Class 1 meshes with large pores showed less IF, CT, fistula formation, calcification, and bridging than class 2 meshes with small pores. Meshes removed for recurrence showed a lowered collagen 1/3 ratio in 70%. Large pore class 1 meshes showed an improved tissue response and may be considered as favorable to prevent inflammatory side effects. The presence of lowered collagen 1/3 ratio in most of the samples with recurrences stresses the relevance of an intact healing process. Late manifestation of complications demands long-lasting follow-up. © 2013 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 008: 000–000, 2013.

Key Words: mesh, hernia surgery, foreign body reaction, medical device

How to cite this article: Klosterhalfen B, Klinge U. 2013. Retrieval study at 623 human mesh explants made of polypropylene—impact of mesh class and indication for mesh removal on tissue reaction. J Biomed Mater Res Part B 2013: 00B: 000–000.

INTRODUCTION

Textiles for reinforcement of a hernia repair have become standard procedure in surgery. In millions of patients, the biocompatibility of mesh devices has been proven. However, implantation of big pieces of textiles in the abdominal wall seems not to be completely free of any risk. Concerns are supported by pictures of shrunken, degraded meshes at a revision operation,1 the discussion whether weight (heavyweight/weight)2 or porosity of meshes is decisive for the tissue ingrowth,3 and the controversies about a suspected impact of the material on clinical outcome4 sometimes even leading to recalls and legal consequences.5

Meshes with its large contact area always induce a foreign body reaction, started by protein absorption at the surface, attracting local inflammatory cells, for example, macrophages that converge to foreign body giant cells, and mature up in foreign body granuloma around the filaments. The local response is influenced by the individual response of the patient, the indication for explantation, and not least the device with its structural and material characteristics.

In contrast to animal experiments with their short follow up and their lack of human comorbidities or diseases, retrieval studies of human patients cover a long period of implantation, several indications for explantation and all kinds of cofactors seen in human patients. Therefore, meshes explanted from various surgeons for various reasons were collected for a systematic histopathological analysis in order to define the impact of mesh structure on tissue response. In this study, we present the results of 623 explanted mesh samples, all made of polypropylene and have been incorporated for up to 180 months.

MATERIAL METHODS

Between 2003 and 2010, explanted meshes from human patients have been submitted to the institute for pathology, Düren, mainly from Germany, but additionally from many...
other parts of the world. All sections have been stored in formaldehyde. The surgeons provided clinical information such as presence of pain, recurrence, or infection, the type of material, the interval between implantation and explantation, the type of hernia and the kind of procedure, without any further attempt of confirmation.

A complete examination could be finished of 623 devices with sufficient clinical information [mesh material, procedure, localization of the mesh, indication for mesh removal (pain, infection, and recurrence), patients age and gender], all made of polypropylene and 170 could be classified as large pore class 1 mesh (Vipro\textregistered, n = 144 and Ultrapro\textregistered, n = 26) and 453 as small pore class 2 mesh (Marlex\textregistered, n = 141, Atrium\textregistered, n = 145, and Prolene\textregistered, n = 167). The meshes have been explanted in 475 males and 148 females, in the groin area by Lichtenstein procedure (n = 179), TAPP (n = 110), or TEP (n = 58), and for repair of abdominal wall hernia as sublay (n = 152) or onlay (n = 91).

Specimens were studied by light-microscopy (LM). For LM tissue samples were fixed in 10% buffered formalin, embedded in paraffin, and 4 \( \mu m \) sections were stained with hematoxylin and eosin and Elastica van Gieson.

Presence of adhesion (low or high density soft tissue not related to abdominal wall structures attached to mesh structures), fistula formation (chronic or acute inflammation forming a passageway to epithelium-lined organ), enrollment with folding of the mesh (mesh fibers in at least double layers), calcification and bridging of scar tissue (fibrous tissue filling out the entire pore of the mesh but no fat tissue) was noticed.

Collagen type I/III ratio

Collagen I and collagen III were stained by immunohistochemistry. Paraffin sections of 3 \( \mu m \) were placed on slides coated with poly-l-lysine (Sigma, Mo.) and dried overnight in a 37°C incubator. All sections were dewaxed in xylol for 10 min and dehydrated through graded alcohol (100, 96, and 70% alcohol each for 5 min). Endogenous peroxidase activity in the tissue sections was blocked with 0.3% \( H_2O_2 \) in methanol for 15 min and afterwards washed in PBS. This procedure was repeated three times, each for 5 min. After incubation in 1% BSA for 30 min, samples were washed in PBS three times each for 5 min. Sections were diluted with primary antibodies at room temperature for 60 min, excessive antibodies were washed off in PBS three times each for 5 min. Mouse antihuman type-I collagen (Sigma-Aldrich, Taufkirchen, Germany, 1:50) and goat antihuman type-III collagen (Sigma-Aldrich, 1:50) were used as primary antibodies. The sections were incubated with secondary antibodies at room temperature for 30 min, jet washed off the excessive antibodies with PBS, and then washed three times each for 5 min. As secondary antibodies, horseradish peroxidase conjugated rabbit antimouse antibody (Dako, Hamburg, Germany, 1:300) for collagen I and rabbit anti-goat antibody (Dako, 1:300) for collagen III were used. As chromogen, 0.06% 3,3-diaminobenzidine tetrachloride was used. Finally, the sections were dehydrated, cleared in xylene, and mounted in Vitro-clud. A counterstain for hematoxylin was performed. The intensity of the antigen–antibody reaction was quantified using the image analyzer system Quantimet 600 (Leica, Germany) with five randomized fields for each specimen. In control sections, primary antibodies were omitted. In all cases, control sections showed no staining under microscopic examination. The background control value was subtracted from the value obtained for each noncontrol section. This was repeated five times for each tissue sample and primary antibody. Results are expressed as ratio of values estimated for collagen type I to type III. In addition, tissue samples were stained with Sirius red. Visualization by crossed polaroid filters allowed estimation of collagen type I, seen as thick yellow, orange or red colored fibers, collagen type III fibers are thinner and stained in pale green shades.

The morphometric evaluation consisted of a quantitative histological analysis of the inflammatory reaction and the soft-tissue reaction. Partial volumes (PV) of tissues were counted in fields of 5 HE slides at a grid of 10 points (100\( \times \); area 0.1 mm\(^2\)) within the interface of 0–300 \( \mu m \). Parameters measured were the percentage share of the area covered by an inflammatory infiltrate (IF; PV%), or by connective tissue (CT; PV%).

Statistics was done by SPSS 18.0 using ANOVA and T-test or Chi\(^2\)-test for comparison of two groups. Results were presented as mean with standard deviation, if not otherwise stated. In case of more groups posthoc Bonferroni was used. A \( p < 0.05 \) was considered as being significant.

RESULTS

For the total cohort of 623 mesh samples the mean period between implantation and explantation was 27\(+/-\)20 month, with a median of 23 month ranging from 0.5 to 180 month (Figure 1). The interval for class 1 meshes was slightly longer with 29\(+/-\)18 month compared with 26\(+/-\)21 months for class 2 meshes (n.s., \( p > 0.05 \)). 25% of the meshes were explanted within 13 months, 50% within 23 months, and 75% within 36 months. In case of infection, the meshes have been explanted after 24\(+/-\)14 month (class 1) or 21\(+/-\)19 month (class 2; n.s.), in case of pain...
after 35+/−21 month and 23+/−15 month (p < 0.05), in case of recurrence after 29+/−18 month and 28+/−23 month, respectively (n.s.). From all meshes, which were explanted because of pain, 75% were taken out from the groin area. Mesh explantation because of infection were done in 68% from the groin, and explantation because of recurrence was done in 61% of the cases from the groin, whereas the rest were extracted from the abdominal wall.

Impact of indication
When analyzing partial volume of IF over time, we found a huge interindividual variation (Figure 2). However, when subgrouping according to indication for explantation there were differences in mean IF. IF was significantly different in the presence of infection (44+/−9) compared with absence of infection (26+/−12), in case of pain (32+/−12) compared with absence of pain (28+/−14), and with recurrence (26+/−12) compared with absence of recurrence (36+/−12; always p < 0.05; Figure 3). Thus IF was higher in the presence of infection or chronic pain, but lower in patients with hernia recurrence.

IF generally was significantly (p < 0.01) and positively related to partial volume of CT with a Pearson’s correlation coefficient r = + 0.368 (Figure 4). Furthermore, IF showed positive correlations with infection (r = +0.540), and pain (r = +0.108) as indication for mesh explantation, the presence of adhesions (r = +0.248), with fistula (r = +0.279), female gender (r = +0.123), and bridging (r = +0.455). Furthermore we found significantly (p < 0.01) negative correlations with time (r = −0.270), presence of recurrence (r = −0.379), folding (r = −0.187), and lowered collagen ratio (r = −0.376), but no significant correlation to patients age or the procedure used for mesh implantation.

Impact of mesh class
Far most of the meshes were explanted because of recurrence: 132 of 170 large pore class 1 meshes (78%), and 278 of 453 small pore class 2 meshes (61%; p < 0.05; Table I). From totally 170 class 1 meshes, only 12% (21) have been explanted because of infection, whereas this was the reason in 22% (98) of the 453 class 2 devices (p < 0.05). Totally, 170 meshes were explanted in the presence of chronic pain, 18 patients (11%) from all class 1 meshes but 152 (34%) from all explanted class 2 meshes (p < 0.05).
confirms the improved tissue integration by large pore class able overlap, but differences in the mean values of IF calcification, bridging, IF, and CT, respectively. Although these differences may indicate different risks and complications in dependency of the mesh class it has to be considered that the meshes did not derive from a homogenous group of patients and procedures. For example, we got almost similar number of class 1 meshes from recurrent incisional hernia (77 class 1 versus 85 class 2), whereas for repair of groin hernia, recurrent groin hernia, and incisional hernia class 2 had been used at least 3 times more often than class 1 meshes. Correspondingly, the meshes of class 1 were neighbored more often to muscles than to fat (71%), whereas class 2 were located similarly in muscle and fat tissue (each 50%; p < 0.05).

However, comparing class 1 and class 2 meshes demonstrated differences in macroscopical as well as in microscopical appearance (Table II). A fistula was seen in 30 patients, 3 with class 1 mesh but 27 with class 2 mesh (p < 0.05). Large pore class 1 meshes showed more folding but less calcification, bridging, IF, and CT, respectively.

Separate analysis for the five meshes reveal a considerable overlap, but differences in the mean values of IF confirms the improved tissue integration by large pore class 1 meshes (Vypro<sup>®</sup> and UltrPro<sup>®</sup>; Figure 5).

**Impact of mesh class on collagen I to III ratio**
Over all collagen I to III ratio was found to be normal in 285 patients (46%), whereas considerably reduced in 338 patients (54%). In the presence of infection, a pathological ratio was seen only in 27% of the samples or in case of chronic pain in 30%, respectively, whereas in patients with a recurrent hernia a lowered collagen I to III ration was detected in 70% of the samples (Figure 6).

Furthermore, the mesh class seemed to influence the collagen I to III ratio as well. In the absence of recurrence only 18% of class 2 meshes but 50% of class 1 meshes had a lowered collagen ratio (p < 0.05), whereas in the subgroup of patients with recurrence 64% (n = 177) of class 2 meshes and even 83% (n = 110) class 1 meshes had a lowered collagen I to III ratio (p < 0.05; Figure 7).

**DISCUSSION**
Tissue response to meshes is defined by lots of confounders, as by any present local complication, the type of procedure and location of the mesh, and last not least the kind of material (i.e., mesh class used).

In this retrieval study of explanted hernia meshes, to our knowledge the biggest in literature, we can demonstrate that 75% of the mesh explants were performed within 3 years after implantation. Despite huge interindividual variations the inflammatory reaction is intensified by infection but not by chronic pain or recurrence. Large pore class 1 meshes in comparison to small pore class 2 meshes showed relatively less inflammation, less often infection or chronic pain as indication for explantation. A disturbed collagen type I / type III ratio was found at the periprosthetic scar tissue in 70% of patients with a recurrence, indicating the presence of a fundamental biochemical reason for the relapse.

In our cohort, the median period until manifestation of infection was 2 years, which indicates either a reactivation of biofilm forming colonies or a de novo settlement of bacteria on the foreign body surface. However, the latest manifestation was seen after 88 month. This is considerably longer than described by Taylor et al. with a clinical manifestation after a median of 4 months,<sup>9</sup> or by Hawn et al. with a median of 7.3 month.<sup>5</sup> The long interval may indicate that the samples collected in this study at an institute for pathology may be a selection of chronic late onset complications rather than early postoperative problems. In some cases, explantation may be the final result after several failing attempts of conservative therapies. However, 25% of

![Image](https://via.placeholder.com/150)

**FIGURE 4.** Partial volume of IF in relation to partial volume of CT of 623 explanted polypropylene meshes, explanted because of infection, pain, or recurrence.
Explants were done more than 3 years after implantation, which means a permanent risk as long as a foreign body stays in the tissue! Interestingly, the explantation for chronic pain similarly was also done after a median of 22 months with the latest explantation 96 months after implantation. Chronic pain was reported in 170 of the 623 patients, from which 75% had their mesh placed into the groin area and only 27% in the anterior abdominal wall. Thus, sometimes chronic pain may develop late over time, mainly if placed in the groin. Chronic pain after a Lichtenstein mesh was seen in 65 cases and after TAPP/TEP in 57 cases. However, as this analysis is done at a highly selected group of patients, it just can be concluded, that none of these two approaches is completely free from this complication or that in this inhomogeneous cohort a significant difference did not result.

The IF around the filaments of a mesh forming a foreign body granuloma persists over years, as already described in a much smaller retrieval study. Expectedly, a marked inflammation with high IF was positively related with infection, adhesion, and fistula, but surprisingly with female gender, as well. This finding may be caused by the differences between the 475 male and 148 female cases with significantly more explantations for infections and less explantations for recurrences in the females. However, any impact of gender on the clinical outcome has to be carefully examined in further studies. As expected, the IF is negatively related to time ($r = -0.27$), but also negatively related to collagen ratio meaning that intense inflammation is linked to predominance of collagen type I, whereas little inflammatory reaction is related with a lowered collagen I to III ratio. However, considering the complex regulation of collagen deposition and collagen maturation any causal interpretation has to be done with care.

Several animal experiments had clearly shown that material characteristics influences the tissue response. Considering the more than 200 different mesh devices on the market every evaluation of mesh specific reactions is increasingly difficult; unless a grouping of meshes is done. Amid proposed in 1997 a grouping focused on risk for
infection, which is based on porosity and differentiates between large (>75 μm) and small pore structures (<75 μm). However, his classification does not consider the modern large pore class 1 meshes, nor did he look at the differences of tissue reaction and integration. In many studies, pore size has been proven to be of major importance to predict the fibrotic reaction and the risk of scarring bridging of the area between the filaments. In preparation of the upcoming hernia register of the European hernia society to enable future mesh related analysis, and with joint agreement of major mesh manufacturers in Germany, the devices were grouped into a Class 1 that was defined as large pore mesh construction, usually pore size of >1 mm with a textile porosity of >60% and an effective porosity of >0%, whereas class 2 meshes were defined as small pore construction (<1 mm) with a textile porosity of less than 60%. Fortunately, in contrast to all uniaxial mechanical characterization porosity has the advantage not to be affected by the inherent anisotropy of the mesh structures. Despite large variations of the individual response we could confirm significant differences between large pore class 1 and small pore class 2 meshes. Large pore class 1 meshes showed less calcification, less bridging, less CT, and less IF. In particular, the latter clearly justifies the dividing of porous meshes into these two groups.

Whereas class 1 meshes were rarely explanted for infection or chronic pain, the main reason for explantation was recurrence. In comparison to samples without recurrence, significantly more patients with mesh explantation due to recurrence showed a decreased collagen I to III ratio. The high rate of altered collagen ratio in the presence of recurrence confirms the assumption of a basic problem of wound healing as reason for recurrences, rather than just being the result of technical failures. Interestingly, this was found even more often with class 1 meshes (83 vs. 64%) and may indicate either that at least the two class 1 devices did not improve the local quality of collagens at the interface to the mesh, or that patients with normal collagen I to III ratio more often develop a recurrence (for technical reasons) if they received a class 2 meshes, or simply that class 1 meshes are used more often in patients with high risk for recurrence.

In a long term analysis of 1,346 elective incisional hernia repairs, 69% with mesh repair (n = 928), and a median follow-up of 73.4 months Hawn et al. reported of 23 mesh removals (2.5%), and 7 enterocutaneous fistulas (0.8%). In another study, they reported of 55 (5.1%) explants out of 1,071 mesh repairs, after a median period of 7.3 months (interquartile range 1.4–22.2). Infection was found to be the most common reason for explantation (69%). Although some very late complications may be missed, correspondingly, an overall a rate of up to 5% of mesh explantation because of infection should be expected in the field of incisional hernia. Additionally, as recurrences may appear in a rate of up to at least 10%, revision in some of these patients may add some more explanted devices, resulting in an assumed rate of 5–10% of all meshes used for repair of incisional hernia to become explanted.

In the groin area, Stremitzer et al. reported on thirty-one of 476 (6.5%) patients who developed a deep surgical site infection after inguinal hernia repair requiring in 45% removal of the implanted mesh graft, the rate of infection apparently should be lower in the groin, and therefore the rate of consecutive mesh explantation, which may be in a range of maximum 1–2% of all meshes. About 0.6–6% of the patients with groin hernia repair furthermore may develop debilitating chronic pain and for this reason may need mesh removal. As long term outcome has to consider about 5 to 10% of recurrences, some more retrieved meshes may be added at the occasion of the repair of a recurrence. Conclusively, considering that a rate of 5% for all meshes become explanted, the 623 meshes of this study reflects only a very small share of explanted meshes (considering more than 1 million mesh implantations in the 7 year period of mesh collection in Germany), but the huge majority likely ended up in some rubbish or some archives of pathology. Despite the clinical data provided by the
explanted mesh materials, even though it is a selection of explanted surgeon were not checked and therefore may be somehow regarded as suspicious, this study clearly illustrates what we can learn from a systematic collection of explanted mesh materials, even though it is a selection of mesh failures. Further efforts will have to address the superposing influence of the patients’ individual immunological capability and the impact of technical details of the procedure. In contrast to most clinical trials, this retrieval study is able to cover a long survey of many years, which should be required for any evaluation of medical devices.

ACKNOWLEDGEMENT

This study was funded by institutional support from the University Clinic of the RWTH Aachen and is co-funded by the European Union (European Regional Development Fund - Investing in your future) and the German federal state North Rhine-Westphalia (NRW). No direct or indirect industry support was utilized.

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