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Coated meshes for hernia repair provide comparable intraperitoneal adhesion prevention

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Abstract
Background Laparoscopic incisional hernia repair with intraperitoneal mesh is associated with a certain degree of adhesion formation to the mesh. This experimental study examined the efficacy of several coated meshes for adhesion reduction.
Methods Five commercially available meshes with a layered coating were placed intraperitoneally in rats and followed up for 90 days: polypropylene and polyester meshes, both coated with absorbable collagen (Parietene Composite and Parietex Composite, respectively), and three polypropylene meshes respectively coated with absorbable omega-3 fatty acids (C-Qur Edge), absorbable cellulose (Sepramesh IP), and nonabsorbable expanded polytetrafluoroethylene (Intramesh T1). Uncoated polypropylene and collagen meshs (Parietene and Permacol, respectively) served as the control condition. Adhesions, incorporation, and tissue reaction were evaluated macro- and microscopically. Additionally, the development of the neoperitoneum was examined.*Results* All the coated meshes performed equally well in terms of adhesion reduction. The collagen mesh performed comparably, but the uncoated polypropylene mesh performed significantly worse. The different coatings led to very differing degrees of inflammation. Ingrowth was observed only at the place of suture but was comparable for all the meshes except C-Qur Edge, which showed the weakest incorporation. Development of a neoperitoneum on the mesh surface occurred independently of whether an absorbable or nonabsorbable coating or no coating at all was present.
Conclusions Commercially available meshes with a layered coating deliver comparable adhesion reduction. The physical presence of a layered coating between the intraperitoneal content and the abdominal wall seems to be more important than the chemical properties of the coating in adhesion formation.

Keywords Adhesion reduction · Coated meshes · Incisional hernia repair · Intraperitoneal adhesion

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Incisional hernias remain a frequent and significant problem in abdominal surgery [1]. Repair of these hernias should include mesh placement whenever possible to reduce recurrence rates compared with suture repair [2]. Laparoscopic repair is preferred over open repair because of a shorter hospital stay, fewer infections, and better recognition of multiple herniations [3, 4].

In case of laparoscopic repair, the mesh is positioned intraperitoneally and becomes a specific lead point for adhesion formation. Adhesions are clinically relevant because they can lead to chronic pain, bowel obstruction,

fistula, and inadvertent enterotomies at reoperation [5–8]. Three in four surgeons therefore aim to prevent adhesions in intraperitoneal mesh implantation [9].

It is believed that adhesion prevention can be achieved by rapid restoration of a continuous mesothelial cell layer on top of a mesh (i.e., neoperitonealization) [10]. To support the formation of such a neoperitoneum, both the chemical composition and the morphology of the mesh have been identified as important contributors [10, 11]. However, only limited data on the development of the neoperitoneum in relation to adhesion formation are available. Furthermore, the influence of the abdominal wall on adhesion formation has been virtually unstudied, although unlike bowel, it is the only structure continuously in close contact with the mesh.

For this study, we hypothesized that differences in the chemical compositions of the mesh coatings provide different outcomes in terms of adhesion formation. To investigate this, meshes were implanted intraperitoneally in rats and followed up for 90 days. In addition, we studied the influence of the abdominal wall and the development of the neoperitoneum on adhesion formation.

Methods and materials

Male Wistar rats weighing 275–325 g had free access to water and food. The experimental protocol complied with the Dutch Animal Experimentation Act and was approved by the local Animal Ethics Committee of Maastricht University, the Netherlands.

Experiments

Experiment 1

The first experiment studied the long-term follow-up evaluation of adhesion formation with five meshes that had a continuous coating and two meshes that had no coating (10 rats per group) during a 90-day follow-up period (Table 1). Each rat received one piece of mesh (3 × 2 cm). The coated meshes were placed with the coatings facing the viscera. The meshes overlapped the midline incision and were secured to the abdominal wall using four single polypropylene 4/0 sutures (Prolene; Ethicon, Johnson & Johnson, Somerville, NJ, USA), one in each corner of the mesh. When the animals were killed, incorporation strength also was assessed.

Experiment 2

The second experiment studied the influence of the abdominal wall on adhesion formation. Two meshes (each 3 × 1 cm) were implanted intraperitoneally in seven rats. The polypropylene mesh without coating (control condition) was placed on one side of the midline, and the coated polypropylene mesh (Intramesh T1) was placed the other side. The coating was placed upside down (i.e., facing the abdominal wall). This excluded the interaction between the abdominal wall and the polypropylene mesh. Intramesh T1 was chosen because it showed one of the least inflammatory reactions in experiment 1. The follow-up period was 16 days, and the positions of the meshes were alternated after each procedure.

Experiment 3

The third experiment studied the early host reaction to three of the aforementioned meshes and the development of the neoperitoneum at a follow-up evaluation after 1, 4, 8, and 16 days. A total of 36 rats (3 rats per group per time point) received either a polypropylene mesh with no coating (Parietene), a polypropylene mesh with an absorbable coating (Sepramesh IP) or a polypropylene mesh with a nonabsorbable coating (Intramesh T1) using the same procedures as in experiment 1.

Operative procedures

The animals were anesthetized with isoflurane 2.5 %. After the abdomen had been shaved and the skin disinfected with 1 % iodine, the abdomen was accessed through a 4-cm midline incision. Subsequent to mesh implantation, the muscular layers were closed with a running suture of polyglactin 4/0 (Vicryl; Ethicon, Johnson & Johnson), and the skin was closed with an intracutaneous running suture of polyglecaprone 4/0 (Monocryl; Ethicon, Johnson & Johnson). No antibiotics were administered, and the procedures were performed under sterile conditions.

The rats were killed with an overdose of carbon dioxide by inhalation. Afterward, the abdomen was opened through an U-shaped incision extending lateral and caudal to the meshes. Thereafter, meshes were scored macroscopically by two blinded and independent observers and excised together with the underlying abdominal wall. In case of disagreement between the observers, a third observer scored the mesh as well, and the median was recorded.

Adhesions

After opening of the abdomen, a standardized picture of the mesh was taken for computer quantification of the extent of adhesions. Furthermore, we recorded the total adhesion score (range, 0–11) based on the extent (0 = no adhesions, 1 = 1–25 % involvement of the mesh surface, 2 = 26–50 % involvement, 3 = 51–75 % involvement, 4 = 76–100 % involvement), type (0 = no adhesions,

Table 1 Overview of the meshes used in this study

Mesh	Basic material	Surface modification	Manufacturer
Parietene	Polypropylene	None	Covidien, Mansfield, MA, USA
Parietene Composite	Polypropylene	Collagen-based layer, absorbable	Covidien, Mansfield, MA, USA
Parietex Composite	Polyester	Collagen-based layer, absorbable	Covidien, Mansfield, MA, USA
C-Qur Edge	Polypropylene	Omega-3 fatty acids layer, absorbable	Atrium, Hudson, NH, USA
Sepramesh IP	Polypropylene	Carboxymethylcellulose and hyaluronic acid fibers layer, absorbable	Bard, Covington, GA, USA
Intramesh T1	Polypropylene	Expanded polytetrafluoroethylene (ePTFE) layer	Cousin, Wervicq-Sud, France
Permacol	Cross-linked collagen	None	Covidien, Mansfield, MA, USA

1 = filmy, 2 = dense, 3 = capillaries present, 4 = larger vessels), and tenacity of adhesions (0 = no adhesions, 1 = adhesions fall apart easily, 2 = traction required, 3 = sharp dissection required) [12, 13].

Incorporation

The mesh surface incorporated into the abdominal wall immediately after the animals were killed was scored as follows: 0 (no incorporation), 1 (1–25 % incorporation of the mesh surface), 2 (26–50 % incorporation), 3 (51–75 % incorporation), and 4 (76–100 % incorporation). Additionally, a piece of mesh overlapping the abdominal wall exactly 1 cm^2 was prepared, and the fixating suture in this region was cut.

Incorporation strength was defined as the maximum tensile strength needed to tear the mesh of the abdominal wall at a constant uniaxial pulling rate of 1 mm/s. A digital tensiometer was used (Advanced Force Gauge; Mecmesin, Slinfold, UK).

Histology

Specimens were fixated in formaldehyde 4 %, dehydrated, and embedded in paraffin. Tissue sections 4–5 μm thick were cut and hematoxylin-eosin stained. Additionally, standard immunohistochemistry was performed for macrophages (monoclonal mouse anti-rat CD68, clone ED-1, 1:50; Hycult Biotech, Uden, the Netherlands) and functional microvilli of mesothelial cells (monoclonal mouse anti-human mesothelial cells, clone HBME-1, 1:50; Dako,Glostrup, Denmark).

An experienced animal pathologist evaluated all the sections. A semiquantitative score was assigned as follows: not present, slightly present (only directly around mesh material), moderately present (also between mesh material), or abundantly present (thick layer of reactive tissue around all mesh material) [14].

Statistical analysis

All data are expressed as means ± standard error of the means. Comparisons were calculated using one-way analysis of variance and *t* tests. A Bonferroni corrected *p* value lower than 0.050 was considered statistically significant. All analyses were performed using SPSS version 17.0 (SPSS, Chicago, IL, USA).

Results

All the animals were thriving and well after mesh implantation except for two animals that died immediately after surgery. At the time the animals were killed, no clinical signs of infection such as pus were observed in any animal.

Experiment 1

In general, adhesions consisted of omental fat and formed mainly at the edges of the mesh and at the fixating sutures. All the meshes performed significantly better than the uncoated polypropylene mesh (Parietene) in terms of adhesions at the 90-day follow-up evaluation (Fig. 1A, B). Parietene Composite and Sepramesh showed the least coverage with adhesions, although significantly less compared only with Parietene and Permacol (Fig. 1A). Qualitatively, Sepramesh showed significantly better total adhesion scores than the other meshes except for Parietene Composite (Fig. 1B).

The strength of fixation was comparable among all the meshes except C-Qur Edge, which showed significantly weaker incorporation (Fig. 1C). However, the mesh surface

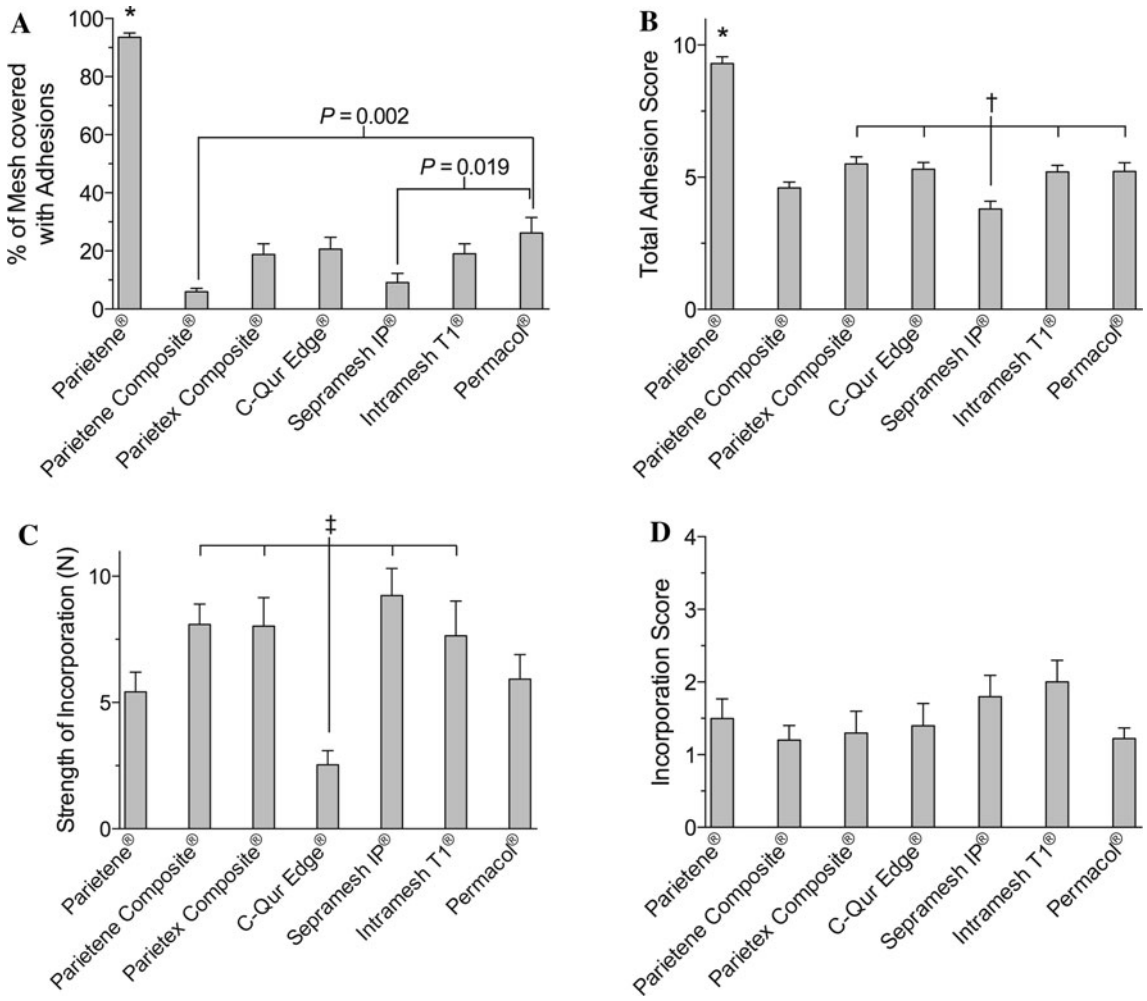

Fig. 1 Comparisons of meshes at the 90-day follow-up evaluation with respect to **A** the percentage of surface covered with adhesions, **B** the total adhesion score, **C** the strength of incorporation, and **D** the incorporation score. The results are expressed as the mean ± standard error. $^{*}p < 0.001$ compared with the other meshes. $^{\dagger}p < 0.05$ compared with Sepramesh. $^{\ddagger}p < 0.05$ compared with C-Qur Edge

incorporated in the abdominal wall was comparable among all the meshes but was restricted mainly to the site of the fixating sutures (Fig. 1D). In addition, two clear linings of functional mesothelial cells were found between the mesh and the abdominal wall except at the suture sites (Fig. 2A).

Permacol showed the least inflammatory reaction and very limited ingrowth of cells, with only minimal amounts of connective tissue surrounding the mesh (Fig. 2B, Table 2). Parietene and Intramesh T1, the two synthetic meshes with no absorbable content, showed only a minimal inflammatory reaction, especially in terms of macrophages.

The coatings of Parietene Composite, Parietex Composite, and Sepramesh IP were no longer present. With Sepramesh IP, moderate amounts of macrophages and some foreign-body giant cells still remained. This was in contrast to Parietene Composite and Parietex Composite, which showed a very active inflammatory reaction with many macrophages and foreign-body giant cells (Fig. 2C). With C-Qur Edge, the coating was still present and surrounded by many macrophages but almost no foreign-body giant cells (Fig. 2D).

Experiment 2

When Intramesh T1 was placed upside down (i.e., with the polypropylene mesh facing the viscera while separated from the abdominal wall by the ePTFE coating), significantly fewer adhesions formed than with the uncoated polypropylene mesh (67 vs 95 %, respectively; $p < 0.001$).

Experiment 3

Already after 1 day, a rich inflammatory infiltrate had formed within the abdominal wall and between the mesh fibers. In the cases of Intramesh and Sepramesh, this infiltrate was covered by the coating but extended beyond

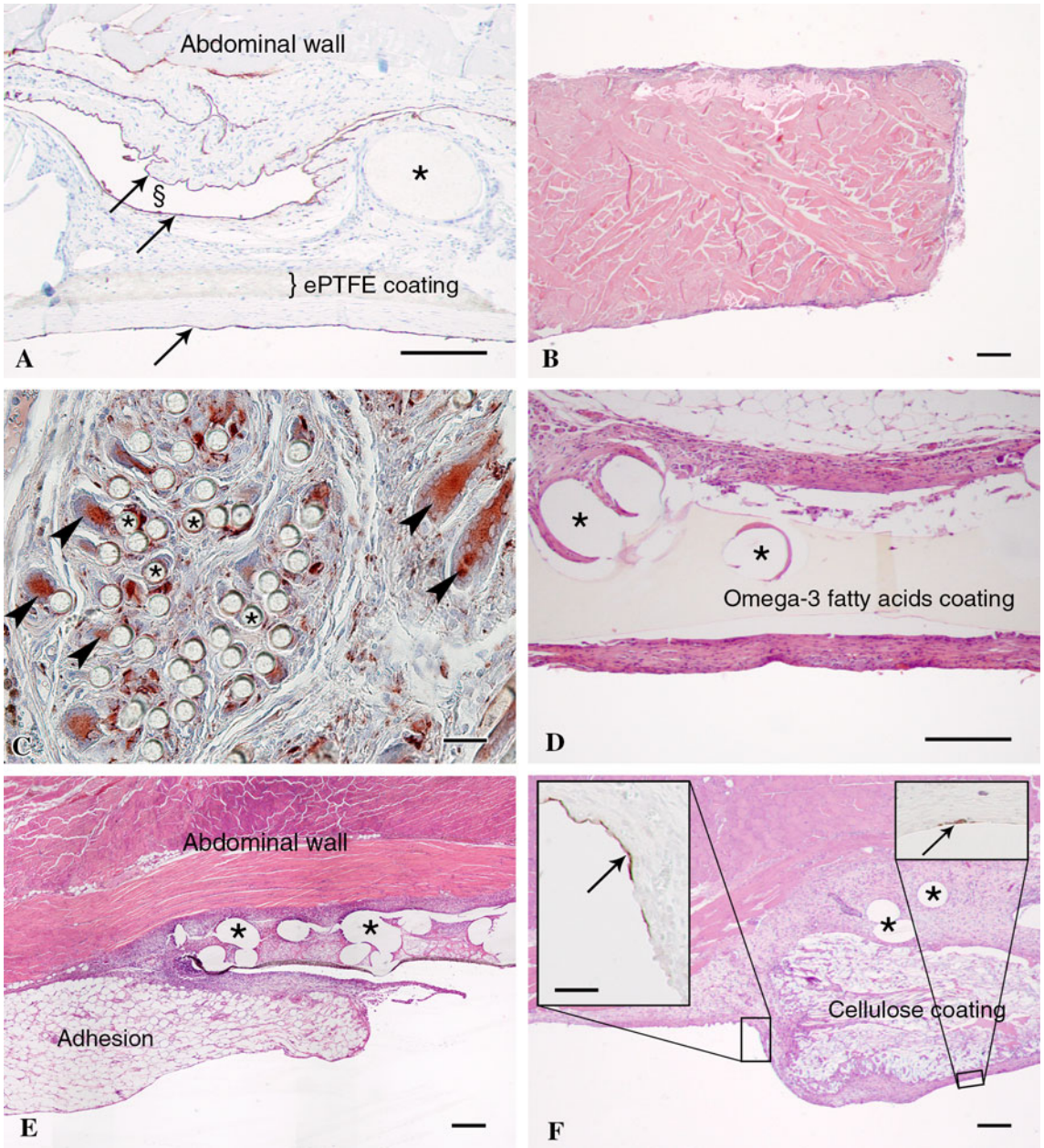

Fig. 2 Representative histologic samples, with *asterisks* indicating mesh fibers. **A** Intramesh T1 at the 90-day follow-up evaluation (original magnification, ×100; anti-HBME-1). Mesothelial cell linings (*arrows*) were found on top of the coating as well as between the abdominal wall and the mesh§. **B** Permacol (original magnification, ×40; hematoxylin and eosin [H&E]) was surrounded by only a very thin layer of connective tissue with no significant infiltration at the 90-day follow-up evaluation. **C** Parietex Composite at the 90-day follow-up evaluation (original magnification, ×200; anti-ED-1) with macrophages merged into foreign-body giant cells (*arrowheads*) surrounding the mesh fibers. **D** C-Qur Edge at the 90-day follow-up valuation evaluation (original magnification, ×100; H&E) shows a still present omega-3 fatty acids coating. **E** Intramesh T1 at the 16-day follow-up evaluation (original magnification, ×40; H&E) with adhesion tissue at the mesh border. **F** Sepramesh IP at the 8-day follow-up evaluation (original magnification, ×40; H&E). The *insets* are from the consecutive slide (original magnification, ×200; anti-ED-1) and show the beginning of a mesothelial cell layer (*arrows*) at the mesh border and on top of the mesh (*bars* indicate 200 μm, except in **C** and in indents of **F**, at which they indicate 50 μm)

the borders of the coating. At those edges, it was associated with adhesion formation (Fig. 2E, F).

With Sepramesh IP, an extensive swelling of the coating appeared after about 4 days of follow-up evaluation together with a decrease in adhesions. Then, rapid infiltration and degradation of the coating paralleled an increase in adhesions from day 8. This was in contrast to Parietene and Intramesh T1 (both consisting of only

Table 2 Histologic evaluation of the meshes at the 90-day follow-up evaluation

	Granulocytes	Macrophages	Foreign-body giant cells	Connective tissue
Parietene	−/+	−/+	+	+
Parietene Composite	+	++	++	+
Parietex Composite	−/+	+++	+++	++
C-Qur Edge	−	++	−/+	++
Sepramesh IP	−/+	++	+	+/++
Intramesh T1	−/+	+	+	++
Permacol	−	−/+	−	−/+

−, absent; +, slightly present; ++, moderately present; +++, abundantly present

nonabsorbable materials), which showed an increase in inflammation and adhesions at about day 4, followed by a slow decrease in inflammation. In the case of Intrasmesh T1, this coincided with a decrease in adhesions, whereas in the case of Parietene, it did not.

With regard to neoperitonealization, functional mesothelial cells were noted mainly at the mesh borders together with some spots on top of the meshes (Fig. 2F). At 16 days, a continuous lining of functional mesothelial cells was clearly identified on top of all overlying tissue, whether adhesions were present or not and irrespective of the type of mesh. Notably, the parietal peritoneum beneath the mesh regained full functional mesothelial coverage on day 16 as well.

Discussion

Many coated meshes are commercially available for laparoscopic intraperitoneal hernia repair. Adhesion prevention and mesh incorporation are considered some of the key features in choosing the best mesh. This experimental study showed that commercially available synthetic meshes with different coatings performed equally well in terms of intraperitoneal adhesion prevention during a 90-day follow-up period. Incorporation also was comparable except with C-Qur Edge.

Both the chemical composition and the morphology of meshes have been attributed a main role in adhesion prevention [10, 11]. The different chemical compositions of the coatings in this study led to marked differences in the inflammatory reactions during a 90-day follow-up period. However, these differences did not lead to any significant differences in adhesion formation. This is an interesting finding that identifies the mere presence of a layered coating as the most important factor in reducing intraperitoneal adhesions. It can be hypothesized that the anti-adhesive effect is due to shielding of the inflammatory infiltrate between the abdominal wall and the mesh fibers (as discussed later). In addition, the surface of the layered coatings is smooth and does not provide any physical anchor points for adhesive tissue as occurs with uncovered mesh fibers.

Sepramesh IP showed a low extent of adhesions, the best qualitative adhesion score, and good incorporation, as already confirmed in other studies [15, 16]. Histologically, we found that the early swelling of the coating burst off the early-formed adhesions. However, the inflammation in the following days caused degradation of the coating together with a small increase of adhesions.

Parietene Composite and Parietex Composite performed comparably well. Regarding Parietex Composite, we and others had already established good experimental and clinical results [14, 17, 18]. Surprisingly, however, a very intense inflammation was noted for both meshes at the 90-day follow-up evaluation, a finding recently reported by others as well [19]. Given the absence of such reaction with bare polypropylene and the fact that both meshes are coated with collagen, this reaction must be ascribed to the collagen coating. Importantly, adhesions were not increased after 90 days in either group. This also might be explained by a thicker tissue layer on top of the mesh, which increases the distance from the inflammation to the intraperitoneal interface [20].

At the 90-day follow-up evaluation, Intramesh T1 with the nonabsorbable layer of ePTFE provoked adhesions similar to those of the other meshes coated with an absorbable layer. A major concern with ePTFE is the risk for infection and the associated need for mesh explantation. In this study, no infections were observed with Intramesh T1 or any other type of mesh. Clinically, the infection rates of ePTFE-based meshes are indeed higher after open repair but not after laparoscopic repair [21, 22].

Permacol, a partly cross-linked porcine dermal collagen mesh, was chosen as an extra control condition because it induces only a minimal inflammatory reaction. In this study, Permacol and C-Qur Edge showed the least inflammatory reaction. With C-Qur Edge, the omega 3 fatty acids in the coating are already known for their potential to temper the inflammatory reaction [23]. However despite the low inflammatory state associated with both meshes, adhesion formation was not reduced. On the other hand, the incorporation strength of C-Qur Edge was reduced. In clinical application, this type of mesh should therefore probably be accompanied with extra fixation.

One of the preferential sites for adhesion formation with coated meshes is the mesh border. Close observation of the mesh borders showed that the inflammatory infiltrate originating from the abdominal wall ranged beyond the mesh border and thus the coating. This way, the infiltrate became unshielded from the peritoneal cavity and a lead point for adhesion formation. We showed the relevance of this abdominal wall infiltrate because shielding it (with coated mesh placed upside down) produced significantly fewer adhesions.

In addition, even when the infiltrate is minimal, the omission of a coating produces detrimental results. This was recently illustrated by Fortelny et al. [24] in patients who had a noncoated reticular mesh of polyvinylidene fluoride implanted intraperitoneally. Despite a favorable inflammatory reaction against the material per se, surgical reintervention due to adhesions was required for 5 of 29 patients [25].

A continuous lining of mesothelial cells generally is regarded as protective against adhesions [10]. However, adhesions had developed already on postoperative day 1 between structures that already were physiologically lined with a layer of mesothelial cells (e.g., omentum and abdominal wall). Next, the development of a full microvilli-bearing mesothelial cell lining was completed at 8–16 days of the follow-up period. Interestingly, this was also true in the case of uncoated polypropylene mesh, for which it seemed that a continuous mesothelial cell lining was positioned over the adhesion-free areas and the extensive adhesions alike. It would therefore be a mechanism that occurs later than the mechanisms leading already to these early adhesions. Consequently, the role of physiologic remesothelialization should be scrutinized in terms of early adhesion prevention.

Mesh incorporation strengths did not differ significantly from those of uncoated polypropylene except for C-Qur Edge. The incorporation area was limited mainly to the areas directly surrounding the fixating sutures. This is in line with the finding that mesh incorporation in an acute wound (i.e., fixating suture site) is more robust than in a chronic wound [26]. As a clinical consequence, scraping of the parietal peritoneum and use of a sufficiently high number of fixation methods might improve incorporation strengths.

This study had some shortcomings. First, the meshes were placed via laparotomy in rats, whereas they are intended mainly for laparoscopic use in humans. Nevertheless, all the meshes were implanted under the same conditions, enabling a comparison between the meshes. In addition, the meshes were placed against an intact peritoneum, comparable with laparoscopic surgery.

Second, the meshes were placed free of any significant forces, as would be the case if defects had been present. The lack of tension might have negatively influenced the stimulus for ingrowth.

Finally, the rats used in this study are not known to have any significant collagen disease. Differences in wound healing may exist in patients with a systemic collagen disorder.

In conclusion, when a mesh is chosen for intraperitoneal use, most currently available commercial meshes with a layered coating prevent adhesion formation equally. Choosing a mesh for laparoscopic intraperitoneal hernia repair should therefore be based on other parameters such as costs and mesh handling, among others.

Acknowledgments The meshes were supplied unconditionally by the respective manufacturers, whereas none of these were involved in the study design or analysis of results.

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