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ORIGINAL PAPER

Formation of adhesion after intraperitoneal application of TiMesh: experimental study on a rodent model

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ABSTRACT

Background: After laparoscopic repair of an incisive hernia, intraperitoneal prosthetic mesh, as a foreign material, is a strong stimulus for the development of adhesion, which may be the cause of serious complications. This experimental study compared three different meshes and their ability to prevent the formation of adhesion and shrinkage.

Methods: Ninety rats were divided randomly into three groups: in Group 1 Proceed mesh was implanted, in Group 2 Ultrapro mesh was implanted, and in Group 3 TiMesh was implanted. Mesh samples were fixed as an intraabdominal mesh in the upper part of the abdomen. Ten animals from each group were sacrificed on days 7, 28 and 60 post-surgery. After opening the abdomen, the formation of adhesion was assessed according to the Surgical Membrane Study Group (SMSG) score, the percentage of shrinkage of the mesh was established and inflammatory reaction scored.

Results: The SMSG score for adhesion was statistically significantly higher on all the postoperative days in the Proceed and Ultrapro mesh groups than in the TiMesh group which caused milder inflammatory reaction on 60th day than others meshes. The size of the mesh after 7 days was statistically significantly smaller in the Proceed and Ultrapro groups than in the TiMesh group, but after 60 days it was statistically significantly larger than in the TiMesh group.

Conclusion: The least formation of adhesion was noted in the TiMesh group, in which the highest level of shrinkage was noticed after 28 and 60 days. TiMesh has advantages over the other meshes studied, but a larger size mesh may be recommended for intraperitoneal application.

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Abdomen; adhesion; hernia; laparoscopic repair; mesh; shrinkage

Introduction

After laparoscopic repair of incisional hernia, a prosthetic mesh, as a foreign body, is a strong stimulus for the development of adhesion,[1] especially if it is located intra-peritoneally. Adhesion may cause serious complications, such as chronic pain, intestinal obstruction, and enterocutanic fistulae.[2–5]

Most commercial meshes are made of propylene, which is strong and chemically stable, but causes tenacious adhesion.[6–8] Changes in the size of the propylene mesh and the shrinkage of the polypropylene material may cause a tissue reaction and therefore reduce the formation of adhesion.[9–11] Therefore, in clinical practice various forms of composite meshes are being introduced, consisting of two adherent layers of material, designed for intraperitoneal placement.[12] Proceed consists of propylene and a coating of poly-diaxonanone and cellulose (Interceed, Ethicon, Somerville, NJ).[11] A reduction in adhesion may also be achieved by increasing the pore size.[9] The composite mesh made of prolene-monocryl (residual polypropylene after absorption of a monocryl component – polyglecaprone), has larger pores, in order to improve the bio-compatibility of the synthetic mesh (Ultrapro, Ethicon, Somerville, NJ).[13]

Titanium has strong bio-compatibility,[14] therefore we assumed that TiMesh, due to its inertness and the size of the pores, causes a lower degree of adhesion between the visceral side of the mesh and the adjacent organs, in relation to the other types of mesh, but there is also less shrinkage of the mesh itself, so it may be the most suitable for intraperitoneal repair.

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Materials and methods

Experimental study

This experimental study was undertaken at the Department of Pathophysiology of the Veterinary Faculty of the University of Sarajevo. The experimental protocol was approved by the Committee of Animal Experiments of the University of Sarajevo. Wistar albino rats, weighing 100–150 g, were kept in standard laboratory conditions before surgery, accommodated in cages and acclimatized to standard laboratory conditions (temperature 20–24°C, 12 h of daylight, 12 h of dark) and all rats subjected to surgery were denied food 24 h beforehand.

Ninety rats were divided randomly into three groups of 30 rats each: Group 1 in which Proceed mesh (Ethicon, Inc.) (polypropylene/polydioxanone) was implanted, Group 2, in which Ultrapro mesh (composite prolene-monocryl mesh with large pores) (Ethicon, Inc.) was implanted, Group 3 in which TiMesh (titanium coated mesh) (PFM Medical, Germany) was implanted.

The surgical procedure

The rats were anaesthetized using ketamine (50 mg/kg). The rats were placed and secured on the operating table in a supine position, with adhesive tape. The animal's abdomen was shaved and disinfected with a solution of povidone and iodine, and dried with a gauze. The laparotomy was performed using a medial incision 4.0 cm long. The implantation of the mesh, 2 cm ×3 cm in size, was undertaken in the upper part of the abdomen, and it was fixed to the peritoneum with prolene 4-0. The incisions were closed using a 3-0 continuous suture. Antibiotic therapy was not used before or after the surgery. During the entire observation period, all the animals were monitored and subject to clinical examination, to test any local or systemic complications.

Parameters of monitoring

Ten animals from each group of subjects were sacrificed on days 7, 28 and 60 post-surgery. Relaparotomy was performed using a left paramedial incision, to gain an overview of the entire abdomen.

Formation of adhesion

After opening the abdomen, the formation of adhesion was assessed according to the Surgical Membrane Study Group score (SMSG score), where each sacrificed animal was assessed jointly by the entire team of researchers (Table 1).

Table 1. Adhesion score according to the Surgical Membrane Study Group.[15]

Adhesion characteristics	Score
Extent of site involment	
None	0
<25%	1
<50%	2
<75%	3
<100%	4
Type	
None	0
Filmy, transparent, avascular	1
Opaque, translucent, avascular	2
Opaque, capillaries present	3
Opaque, large vessels present	4
Tenacity	
None	0
Adhesion falls apart	1
Adhesion lyses with traction	2
Adhesion requires sharp dissection	3
Possible total	

Mesh shrinkage

Mesh shrinkage is defined as the relative loss of area in comparison with the original size of the mesh (%). After extracting the mesh, the size of the mesh was measured and the percentage of mesh shrinkage assessed in relation to the initial size of the implanted mesh.

Histology

Bioptical material (part of the tissue with the mesh) was formalin fixed, paraffin embedded, and was cut into standard 5 μm cuts and colored by applying according to the standard procedure for H&E (hematoxylin eosin) staining, and then mounted by using Canada balsam. Microscopic analysis was performed with an Olympus BX41 microscope (Tokyo, Japan). The inflammatory response was graded semi-quantitatively as a mild, moderate or severe inflammation.

Statistical analysis

The results of SMSG score and the size of the mesh are expressed as a mean value with SD, 95% CI for mean, other variables in Med (± range). ANOVA was used to compare SMSG scores, the size of the mesh and inflammatory reactions, while the Kruskal–Wallis test was used to compare other observables in the Proceed, Ultrapro and TiMesh groups. *Post hoc* analysis of SMSG score and the size of the mesh were performed using the Fisher's least significant difference (LSD) *post hoc* test.

Post hoc analysis of other observables was performed by the Mann–Whitney test. To avoid inflation of a Type I error, Bonferroni's correction was applied. We multiplied the level of significance in each *post hoc* comparison by factor 3. All reported *p* values were obtained by using Bonferroni's

Table 2. Results on day 7 post-surgery.

	Extent of site involvement	Type of adhesion	Tenacity of adhesion	SMSG score	Size of mesh
Proceed					
N	10	10	10	10	10
Mean				7.60	18.20
Median	2.00	3.00	3.00		
SD				2.27	1.03
95% CI for mean				5.98–9.22	17.46–18.94
Range	3.00	2.00	1.00	6.00	3.00
Minimum	1.00	2.00	2.00	5.00	17.00
Maximum	4.00	4.00	3.00	11.00	20.00
Ultrapro					
N	10	10	10	10	10
Mean				6.60	18.30
Median	1.50	2.50	2.00		
SD				2.011	1.702
95% CI for mean				5.16–8.04	17.08–19.52
Range	3.00	2.00	1.00	6.00	5.00
Minimum	1.00	1.00	2.00	4.00	15.00
Maximum	4.00	3.00	3.00	10.00	20.00
TiMesh					
N	10	10	10	10	10
Mean				4.60	19.90
Median	1.50	1.50	1.00		
SD				2.37	1.32
95% CI for mean				2.91–6.29	19.67–21.13
Range	2.00	3.00	2.00	7.00	1.00
Minimum	1.00	0.00	0.00	1.00	19.00
Maximum	3.00	3.00	2.00	8.00	20.00

correction. The differences in the measurable quantities were statistically significant if corrected to $p < 0.05$. For statistical analysis, we used the ARCUS Quickstat Biomedical (Cheshire, UK) and MedCalc softwares (Ostend, Belgium).

Results

There were no intraoperative mortalities, complications related to anesthesia or infections of the implanted prosthetic material in the rats. There was one post-operative mortality of a rat in the Ultrapro group, on the 45th day. Upon examination of the abdomen, ileus was established that had been caused by retraction of part of the small intestine between the prosthetic material and the front part of the stomach wall. No post-operative reduction in the in-take of food or liquid was observed. Adhesions were registered in all three groups. The most common adhesions were with the omentum, then with the small and large intestines. The most frequent location of adhesion was any surface in the immediate vicinity of the fixed mesh on the front side of the abdominal wall.

Results on day 7 post-surgery

Using ANOVA, it was established that the SMSG adhesion score differed statistically significantly ($p = 0.017$). Using the LSD *post hoc* test, it was found that the adhesion score was statistically significantly higher in the Proceed group than in the TiMesh group ($p = 0.005$) (Table 2 and Figure 1). In other cases, there was no statistically significant difference in the adhesion score (Proceed-Ultrapro: $p = 0.32$; Ultrapro-TiMesh: $p = 0.05$).

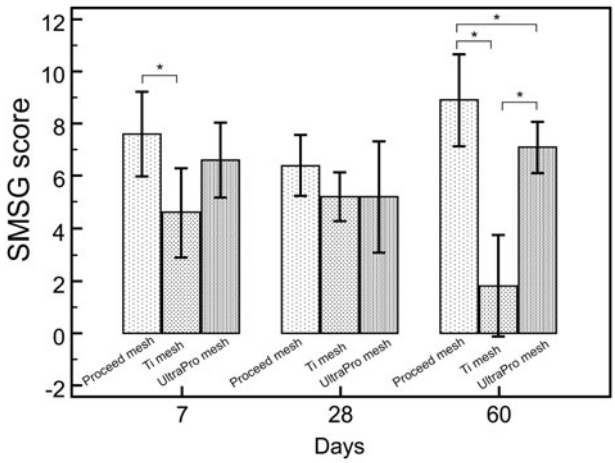

Figure 1. SMSG score on days 7, 28 and 60 post-surgery. *Statistically significant difference.

The extent of site involvement, the type of adhesion

The Kruskal–Wallis test showed that the extent of site involvement did not differ statistically significantly ($p = 0.77$), which is also true for the type of adhesion ($p = 0.06$). There was a statistically significant difference in the tenacity of the adhesion ($p < 0.0001$). *Post hoc* analysis was undertaken

Table 3. Results on day 28 post-surgery.

	Extent of site involvement	Type of adhesion	Tenacity of adhesion	SMSG score	Size of mesh
Proceed					
N	10	10	10	10	10
Mean				6.40	17.90
Median	2.00	2.00	2.00		
SD				1.65	.74
95% CI for mean				5.22–7.58	17.37–18.43
Range	2.00	3.00	1.00	6.00	2.00
Minimum	1.00	1.00	2.00	4.00	17.00
Maximum	3.00	4.00	3.00	10.00	19.00
Ultrapro					
N	10	10	10	10	10
Mean				5.20	19.80
Median	1.00	2.50	2.00		
SD				2.97	.63
95% CI for mean				3.07–7.33	19.35–20.25
Range	3.00	3.00	3.00	9.00	2.00
Minimum	0.00	0.00	0.00	0.00	18.00
Maximum	3.00	3.00	3.00	9.00	20.00
TiMesh					
N	10	10	10	10	10
Mean				1.80	14.80
Median					
SD				2.70	2.82
95% CI for mean				0.00–3.73	12.78–16.82
Range	4.00	3.00	1.00	8.00	10.00
Minimum	0.00	0.00	0.00	0.00	10.00
Maximum	4.00	3.00	1.00	8.00	20.00

using the Mann–Whitney test, with the Bonferroni correction.

Testing for statistical significance of the differences in inflammatory reaction between the three groups after sacrifice on day 7 was performed by ANOVA. There was no statistically significant difference between the three groups in terms of inflammatory reaction ($p < 0.96$).

Tenacity of adhesion

The score for the tenacity of the adhesion was statistically significantly stronger in the Proceed mesh group than in the TiMesh group ($p = 0.0001$). Likewise, the tenacity of the adhesion was greater in the Ultrapro group than in the TiMesh group ($p = 0.003$). No statistically significant difference between the Proceed and TiMesh groups was found ($p = 1.0$).

Results on day 28 post-surgery

Using ANOVA, no statistically significant difference was established in SMSG adhesion scores between the groups ($p = 0.001$) (Table 3 and Figure 1) and there was no statistically significant difference between the three groups in terms of inflammatory reaction ($p < 0.89$).

The Kruskal–Wallis test established that three measured characteristics differed statistically significantly between the groups: the size of adhesion ($p = 0.011$), the type of adhesion ($p = 0.007$), and the tenacity of adhesion ($p < 0.0001$).

Extent of site involvement

The extent of site involvement after 28 days was statistically significantly greater in the Proceed group than in the Ultrapro ($p = 0.035$) and the TiMesh group ($p = 0.017$). The extent of site involvement in the Ultrapro and TiMesh groups did not differ significantly ($p = 0.37$). The same was true for the Proceed and Ultrapro groups ($p = 0.82$).

Type of adhesion

The score for the type of adhesion was significantly higher in the Proceed mesh group than in the TiMesh group ($p = 0.003$). In the Ultrapro group, it was significantly higher than in the TiMesh group ($p = 0.029$). There was no significant difference between the Proceed and Ultrapro groups ($p = 0.82$).

Tenacity of adhesion

The score for the tenacity of the adhesion was significantly stronger in the Proceed mesh group than in the TiMesh group ($p < 0.0001$). In addition, the tenacity of the adhesion was statistically significantly stronger in the Ultrapro group than in the TiMesh group ($p = 0.008$). There was no significant difference between the Proceed and Ultrapro groups ($p = 1.0$).

Table 4. Results on day 60 post-surgery.

	Extent of site involvement	Type of adhesion	Tenacity of adhesion	SMSG score	Size of mesh
Proceed					
N	10	10	10	10	10
Mean				8.90	18.30
Median	2.50	4.00	3.00		
SD				2.47	2.50
95% CI for mean				7.13–10.67	16.51–20.09
Range	3.00	3.00	2.00	8.00	8.00
Minimum	1.00	1.00	1.00	3.00	12.00
Maximum	4.00	4.00	3.00	11.00	20.00
Ultrapro					
N	10	10	10	10	10
Mean				7.10	19.50
Median	3.00	2.00	2.00		
SD				1.37	1.08
95% CI for mean				6.12–8.08	18.73–20.73
Range	3.00	1.00	2.00	5.00	3.00
Minimum	1.00	2.00	1.00	4.00	17.00
Maximum	4.00	3.00	3.00	9.00	20.00
TiMesh					
N	10	10	10	10	10
Mean				1.80	14.80
Median	0.00	0.00	0.00		
SD				2.70	2.82
95% CI for mean				0.00–3.73	12.78–16.82
Range	4.00	3.00	1.00	8.00	10.00
Minimum	0.00	0.00	0.00	0.00	10.00
Maximum	4.00	3.00	1.00	8.00	20.00

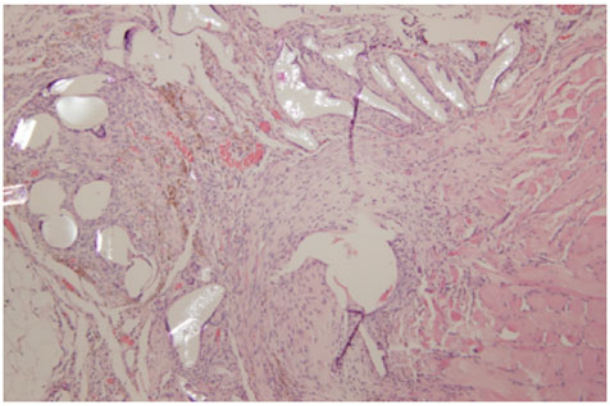

Figure 2. Proceed mesh. Moderate inflammation on day 60 post-surgery.

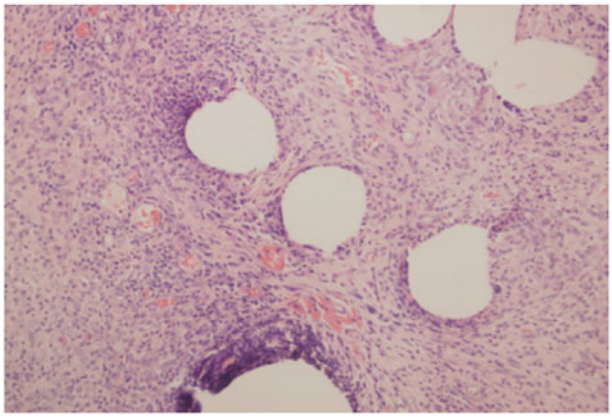

Figure 3. Ultrapro mesh. Moderate inflammation on day 60 post-surgery.

Results on day 60 post-surgery

The SMSG score of the adhesion was statistically significantly higher in the Proceed group than in the TiMesh group ($p < 0.0001$) (Table 4 and Figure 1). No significant difference between Proceed and Ultrapro was found ($p = 0.086$). The adhesion score was statistically significantly higher in the Ultrapro mesh group than in the TiMesh group ($p < 0.0001$).

Statistical analysis of the inflammatory reaction showed that there was a statistically significant difference between the Proceed and the TiMesh ($p < 0.017$), while there was no statistically significant difference between the other groups (Figures 2–4). The inflammatory reaction was milder in the titanium group after 60 days.

The Kruskal–Wallis test established that the three measured characteristics differed statistically significantly between the groups: the extent of site involvement ($p = 0.004$), the type of adhesion ($p < 0.0001$) and the tenacity of adhesion ($p < 0.0001$). Using ANOVA, it was shown that there was a statistically significant difference in adhesion scores between the groups ($p < 0.0001$).

Extent of site involvement

The extent of site involvement was statistically significantly greater in the Proceed mesh group than in the TiMesh group ($p = 0.01$). There was no significant difference between the Ultrapro ($p = 0.95$) and Proceed groups ($p = 0.003$).

Type of adhesion

The score for the type of adhesion was statistically significantly higher in the Proceed group than in

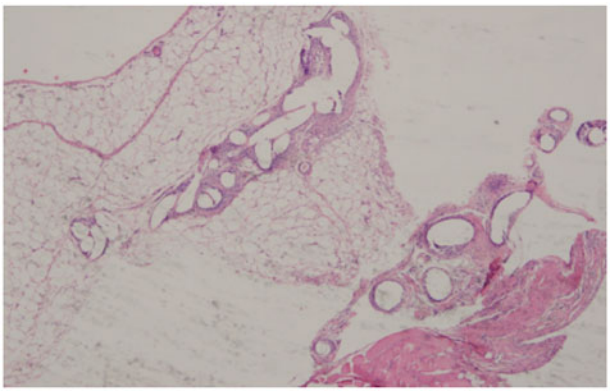

Figure 4. Ti Mesh. Mild inflammation on day 60 post-surgery.

the Ultrapro ($p = 0.003$) and the TiMesh groups ($p < 0.0001$). The type of adhesion was also statistically significantly higher in the Ultrapro mesh than in the TiMesh group ($p = 0.002$).

Tenacity of adhesion

The tenacity of adhesion was statistically significantly stronger in the Proceed group than in the Ultrapro ($p = 0.008$) and the TiMesh groups ($p < 0.0001$). The tenacity of adhesion was also statistically significantly stronger in the Ultrapro mesh group than in the TiMesh group ($p < 0.0001$).

The size of the mesh

Using the ANOVA procedure, it was established that the size of the mesh differed significantly between the groups after 7 days ($p = 0.004$), 28 days ($p < 0.0001$), and 60 days ($p < 0.0001$). *Post hoc* analysis was performed using the LSD *post hoc* test.

The size of the mesh after 7 days was statistically significantly smaller in the Proceed mesh group than in the TiMesh group ($p = 0.003$). The size of the mesh was smaller in the Ultrapro mesh group than in the TiMesh group ($p = 0.005$) (Figure 5). The difference in the size of mesh between the Proceed and Ultrapro groups was not statistically significant ($p = 0.85$).

The size of the mesh after 28 days was significantly smaller in the Proceed mesh group than in the TiMesh group ($p < 0.0001$). In the Ultrapro group, the size of the mesh was statistically significantly larger than in the TiMesh ($p < 0.0001$) and Proceed ($p = 0.02$) groups (Figure 5).

After 60 days, the size of the mesh was statistically significantly larger in the Proceed mesh group than in the TiMesh group ($p = 0.002$). In the Ultrapro group, it was statistically significantly larger than in the TiMesh group ($p < 0.0001$). There were no statistically significant differences between

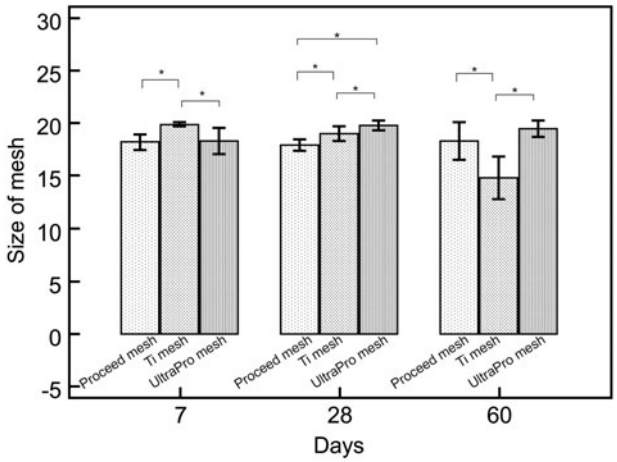

Figure 5. Size of tested meshes on days 7, 28 and 60 post-surgery. *Statistically significant difference.

the Ultrapro and Proceed groups ($p = 0.25$) (Figure 5).

Discussion

Intra-abdominal adhesions may lead to potential complications, and it is necessary to use materials with which the formation of adhesion is eliminated or reduced to the lowest possible extent. Polypropylene causes a strong stimulus for the formation of adhesion.[8] Changing the surface of the propylene mesh and increasing the pore size also affects the formation of adhesion.

In our study, throughout all the days tested, the mesh coated with titanium had the lowest SMGS score. The inflammatory reactions and the formation of adhesion peaked on postoperative day 7.[16] The tenacity of the adhesion and the size of the adhesion were lowest in the group with the titanium coated mesh on all the test days. The inflammatory reaction was milder in the titanium group after 60 days.

Proceed, a reduced-polypropylene-content mesh, may have some benefits over heavyweight poly-propylene Composix or Marlex,[17] but in comparison with meshes with larger pores or titanium coated mesh, it still caused greater formation of adhesion. In the study by Emans [6] which compared Prolene, Proceed, NVP (*N*-vinyl pyrrolidone) and BMA (*n*-butylmethacry-late), the most remarkable adhesions were with Proceed. On days 7 and 30, Proceed was the only mesh surrounded by macrophage cells that contained foreign materials, presumably degradation products of the surface coating. This means that the choice of the right coating is crucial.

Titanium mesh was linked with the least inflammatory reaction with the surface in terms of

duration and scar formation.[7] This may explain why the use of TiMesh is linked with the least subjective sensation of the presence of a foreign body and weather changes.[18]

However, conflicting results also exist. In the study by Burger, who used the rat model, TiMesh and Ultrapro showed extensive adhesion formation 7 days postoperatively.[19]

In fact, the reduction in the inflammatory response, and the formation of adhesion, correlates directly to a reduction in the size of the implanted material.[20] Certainly the chemical composition of the coating also plays an important role in the prevention of adhesion.[21] It is important to point out that low prosthetic loads decrease bacterial adherence [22] as well as chronic pain.[23]

Most mesh materials are subject to some degree of shrinkage after implantation. Mesh shrinkage is linked with a recurrence of the hernia. In this study, shrinkage occurred in all the meshes tested. In addition, the size of the shrinkage in our study on the 7th postoperative day was least in the titanium mesh, but on days 28 and 60 it was the greatest in the titanium group. In the another study, Ultrapro showed the least loss of mesh surface on day 7, but on day 30 there was no significant difference between Prolene, TiMesh, Ultrapro, Proceed and Parietex Composite.[19] The results of other studies are similar; there was no superiority in the mean values of mesh shrinkage between TiMesh, Vypro II, Sepramesh and DynaMesh in rats.[24]

The patho-physiological reactions included in the phenomenon of shrinkage are extremely complex. Mesh shrinkage is in fact the last link in the body's chain of reactions to the foreign material.[10]

Overall, this study suggests that the smallest formation of adhesion in the case of titanium mesh makes it suitable for the intra-peritoneal repair of the abdominal wall, but the size of the mesh is questionable, because the greatest shrinkage was noticed with that mesh, on days 28 and 60, so a larger size mesh should be recommended for repair. The results of this and similar studies need to be confirmed in randomized control studies. The development of new mesh materials assumes a better understanding of the mechanisms of the reaction of the foreign body and the formation of adhesion, as well as mesh shrinkage.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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