MORPHOLOGICAL CHARACTERISTICS OF REPARATIVE PROCESS AFTER INTRAPERITONEAL ABDOMINAL WALL MESH PLASTY DEPENDING ON ENDOPROSTHETIC MATERIAL AND STRUCTURE IN EXPERIMENT

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The aim of the investigation was to study in experiment morphological characteristics of reparative process in the zone of intraperitoneal repair of the abdominal wall by meshes in immediate postoperative period depending on endoprosthetic material and composition.

Materials and Methods. Meshes were implanted in rabbits by open IPOM technique (n=61). Polypropylene (PP Std), polyvinylidenfluoride (PVDF), reperene (R) endoprostheses and composite endoprostheses (PP Std/R, PVDF/R, PP Std/PVDF) were used. The implantation area was analyzed morphologically in 30 days.

Conclusion. The course of reparative process depends on mesh material and structure that should be taken into consideration when choosing a technique for their implantation. The use of standard polypropylene for intraperitoneal plasty without isolation procedure is possible in exceptional cases. PVDF implants have a number of advantages in intraperitoneal implantation and can be extensively used. Reperen endoprostheses are appropriate for temporary closure of the abdominal cavity best of all. The use of composite synthetic meshes with fibrous (mesh) structure of parietal side and complete adhesive cover on visceral side for intraperitoneal abdominal wall plasty is an optimal and morphologically proved approach.

Key words: hernia; mesh; tension-free plasty; IPOM; synthetic endoprostheses.

Prosthetic repair has come to the forefront in surgical management of abdominal hernias that made it possible to improve considerably the results and reduce the recurrence rate by several times [1–4]. The surgery variants are generally distinguished according to the technique of mesh endoprosthesis position, the main ones among them being the following: preperitoneal, retromuscular, supra-aponeurotic and intraperitoneal mesh position [5, 6]. In accordance with on-line poll held at the 8th conference "Topical problems of herniology" (Moscow, Russia, 2011), 42.1% of Russian surgeons use intraperitoneal onlay mesh (IPOM). This method is estimated as simple, easy-to-master and safe [7]. The procedure takes minimum time, indispensible in urgent surgery, especially in patients with high operation and anesthetic risk.

However, an extensive use of IPOM is related to the

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risk of intraabdominal complications. Intraperitoneal endoprostheses are known to be associated with the development of peritoneal commissures in abdominal cavity, acute intestinal obstruction, intestinal fistulas [8–12]. A reparative process in the implantation zone is a matter of importance. The researchers estimate adequate mesh mesothelization and neoperitoneum formation on its surface as an absolute morphological basis for a favorable postoperative course [13]. Otherwise, on visceral implant surface there occur massive collagen disease and the formation of coarse adhesions with intestinal loops.

Up to the present there has not been developed an ideal endoprosthesis for IPOM [14]. There have been suggested tens of various meshes with a number of essential characteristics of composition, structure and weaving [15]. The basic method to study a reparative process in herniology is prosthetic repair modeling in experiments on laboratory animals [16]. Present researchers pay much attention to a detailed analysis of various endoprostheses application [17]. It seems very important that a mesh should provide never-failing connective tissue extension on abdominal wall side, but not resulting in massive adhesive process with visceral organs [13]. It is worthy to note that in late postoperative period there were found critical differences in using standard polypropylene mesh, a number of its improved versions and composite implants [13, 18]. However, there are no such differences in adhesive process at early stages. The mentioned fact is important, since some life-threatening complications can develop just in this period [4]. Despite a considerable amount of experimental and clinical studies, the dependence of a reparative process course on the endoprosthesis structure and its composition has not been yet completely determined.

The aim of the investigation was to study in experiment morphological characteristics of a reparative process in the zone of intraperitoneal repair of the abdominal wall by meshes in immediate postoperative period depending on endoprosthetic material and composition.

Materials and Methods. On the base of Central Scientific Research Laboratory of Scientific Research Institute of Applied and Fundamental Medicine, Nizhny Novgorod State Medical Academy (Russia), we carried out an experimental modeling of intraperitoneal abdominal wall plasty by various synthetic endoprostheses. The work was performed in accordance with ethical principles established by European Convention for the protection of vertebrata used for experimental and other scientific purposes (the Convention was passed in Strasburg, Mar, 18, 1986, adopted in Strasburg, Jun, 15, 2006). The operations (n=61) were made on rabbits (20 animals) under general anesthesia (Nembutal IV, 30 mg/kg). There were used the meshes of standard polypropylene (PP Std, mesh thickness — 500 μm, thread — 120 μm, specific gravity — 62 g/m^2), polyvinylidenfluoride (PVDF, mesh thickness — 480 μm, thread — 120 μm, specific gravity — 160 g/m^2), reperene (R, thickness 300 μm), composite endoprostheses — standard polypropylene and polyvinylidenfluoride (PP Std/PVDF), standard polypropylene and reperene (PP Std/R), polyvinylidenfluoride and reperene (PVDF/R). IPOM technique met the approaches generally accepted in modern herniology, and was in agreement with the statements approved by the conferences of the Russian Hernia Society, and did not differ from that described in standard guidelines and original articles [5, 6, 9, 18].

The animals were sacrificed on day 30 by Nembutal overdose. The results were morphologically analyzed. The preparations were fixed in 10% neutral formaline solution, and filled in the blocks. The sections were prepared on microtome Leica CM 2000R (Germany), hematoxylin-eosin and van Gieson's stained, and studied on a light-optical device (Leica DM 1000, x40–400) with photofixation of images (Leica DFC 290). The findings were comparatively assessed morphologically. The program ImageJ 1.46 (Wayne Rasband, National Institute of Health, USA) was used to make measurements, statistical analysis was made using Kolmogorov–Smirnov test by means of Origin Pro 8 in Windows 8, p-values of <0.05 were considered significant.

Results. A month later the formation of new connective tissue was observed on all preparations. PP Std series appeared to have the maximum number of collagen fibers, and around endoprosthesis elements they formed spiral bundles, fibrages, nodes (Fig. 1, *a*). There was found a significant fibroblast pool, and thick thickness of connective tissue capsule, the fewest number of vessels. The large intestine wall was fixed to the mesh edge (Fig. 1, *b*), the intestine having significant cell infiltration that indicated the presence of a marked inflammatory component (Fig. 1, *c*). There was maximum collagen near this area. Visceral surface beyond the adhesive process zone had collagen areas and neoperitoneum regions (Fig. 1, *d*) with no inflammatory signs, and repair zone was covered by newly formed peritoneum, which was present even in close proximity to the inductor of collagenogenesis — a mesh node. On the other hand, the mesothelization after PP Std implantation was not adequate. The adjoining areas had imperfect, fenestrated neoperitoneum, though that zone had no adhesive process and inflammatory infiltration, and angiogenesis was estimated as being adequate, and collagenogenesis was rather moderate (Fig. 1, *e*).

PVDF series was found to have adequate vascularization, shallow thickness of connective tissue capsule, the absence of inflammatory cell infiltration (Fig. 2). Collagen fibers were located mainly in the direction of one endoprosthesis element to another, and were not arranged in the form of spirals and nodes. Visceral side of the preparation in central zone had mesothelial layer of a small thickness, its periphery having no fenestration areas (Fig. 2, *b*).

The surface of R endoprostheses was covered by thin perfect neoperitoneum (Fig. 3). There was minimal collagenogenesis, no inflammatory infiltration and adhesive process on the side of the abdominal cavity in the implantation zone. However, the endoprosthesis itself was not well fixed to the abdominal tissues, and mesothelial layer was easily separated from the mesh.

Morphological picture of the implantation zone of a knitted composite mesh PP Std/PVDF was mosaic. The surface had adequate mesothelial lining above PVDF fibers. Beneath PP Std threads maximum collagen was concentrated, and above PP threads mesothelium was frequently absent. Sometimes from the visceral side

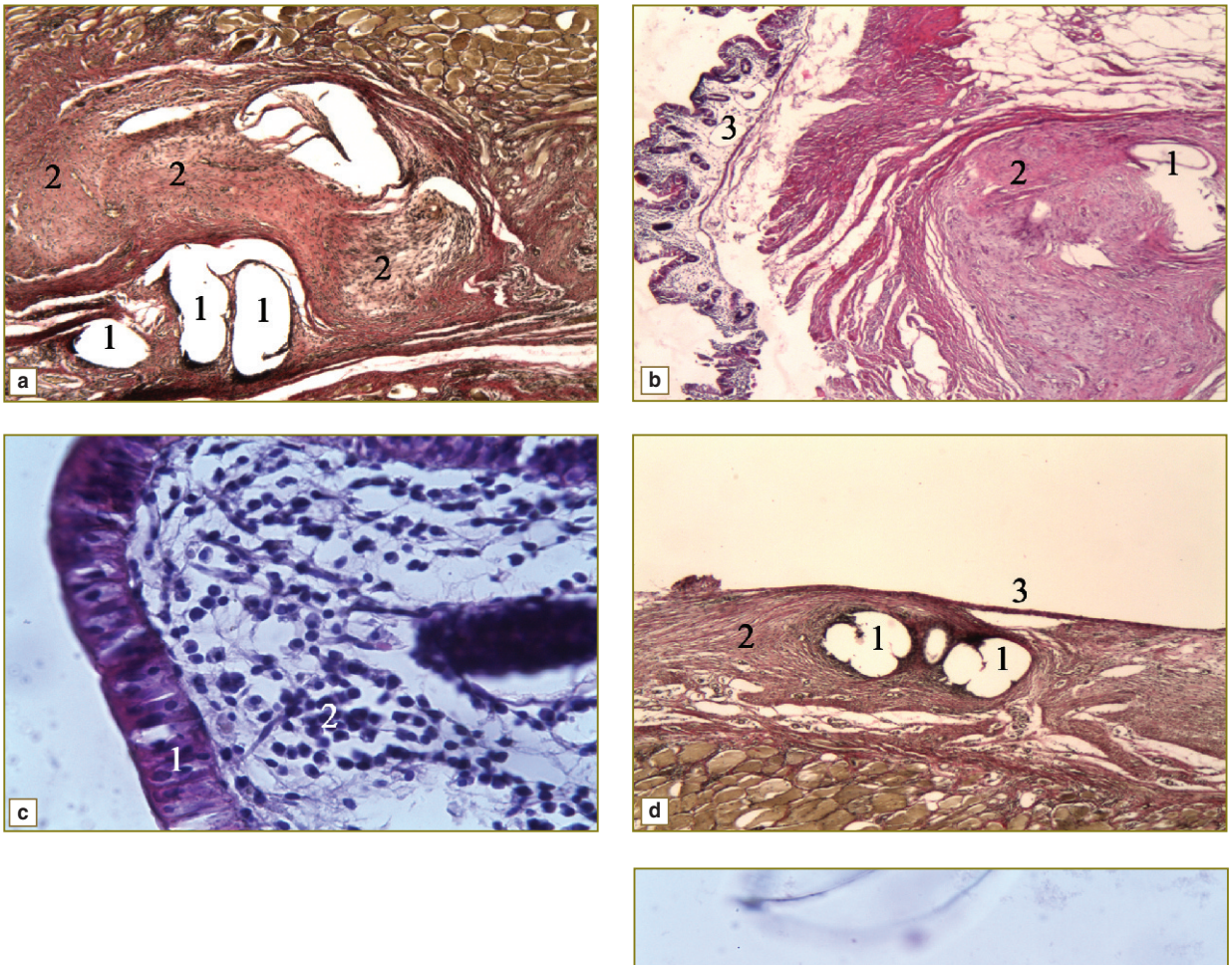

Fig. 1. The repair zone after standard polypropylene implantation: *a* — van Gieson's stain, ×200: *1* — fibers of polypropylene mesh; *2* — connective tissue; *b* — hematoxylin-eosin staining, ×100: *1* — fibers of polypropylene mesh; *2* — connective tissue; *3* — intestine; *c* — hematoxylin-eosin staining, ×400: *1* — intestinal epithelium; *2* — cell infiltration; *d* — van Gieson's stain, ×100: *1* — mesh fibers; *2* — connective tissue; *3* — neoperitoneum; *e* — hematoxylin-eosin staining, ×400: *1* — connective tissue; *2* — a vessel; *3* — neoperitoneum; *4* — neoperitoneum fenestration zone

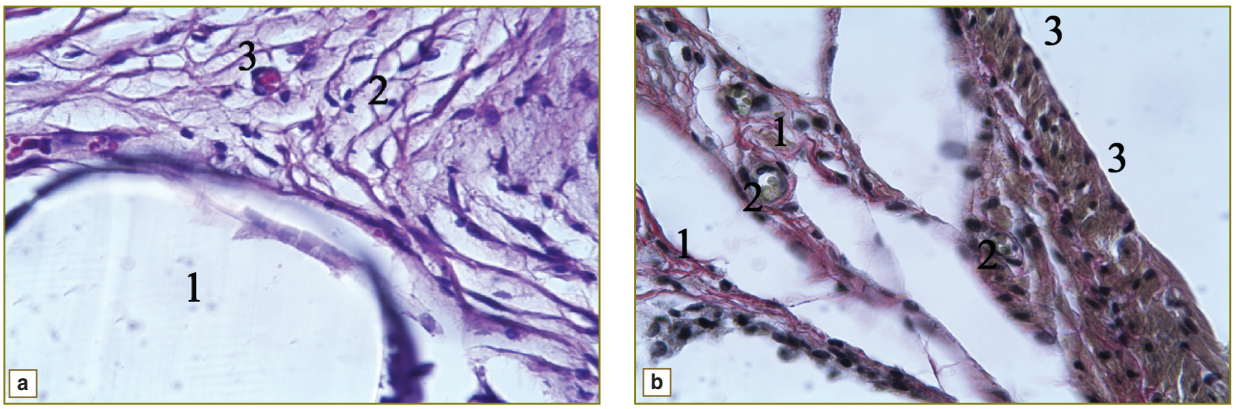

Fig. 2. The repair zone after polyvinylidenfluoride implantation: *a* — hematoxylin-eosin staining, ×400: *1* — a mesh fiber; *2* — connective tissue; *3* — a vessel; *b* — van Gieson's stain, ×400: *1* — connective tissue; *2* — a vessel; *3* — neoperitoneum

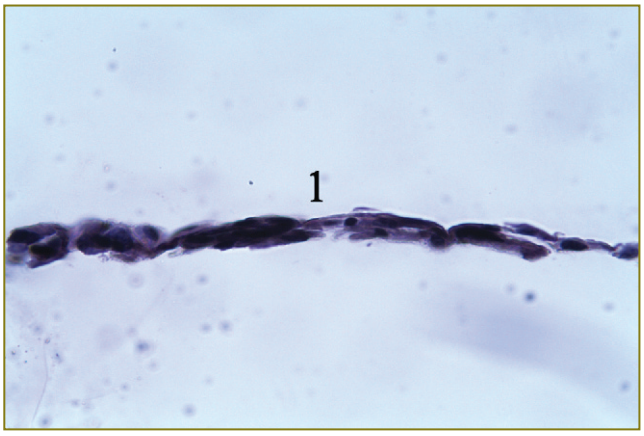

Fig. 3. The repair zone after reperene implantation; van Gieson's stain, ×400: *1* — neoperitoneum

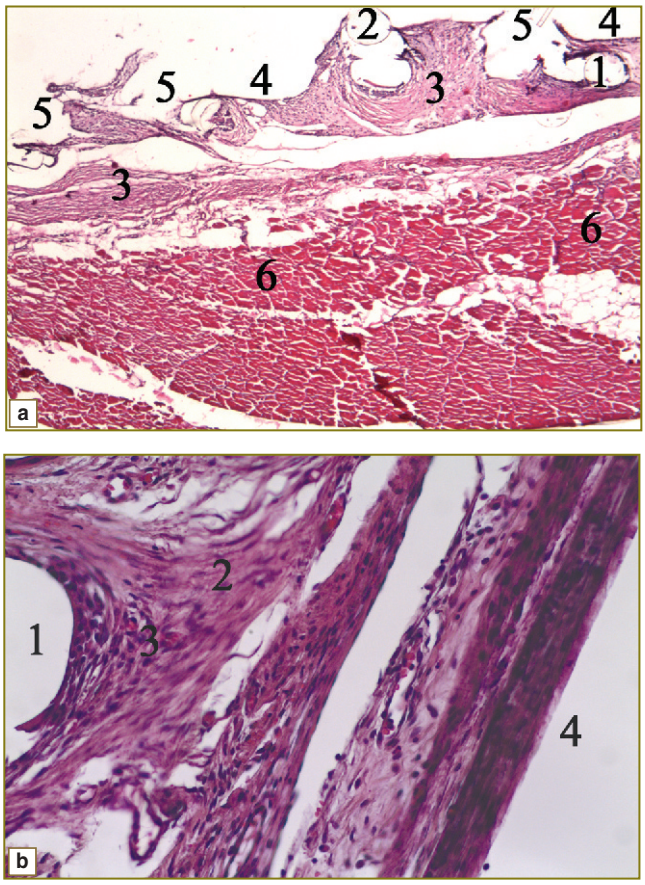

Fig. 4. The repair zone after implantation of composite mesh made of standard polypropylene and polyvinylidenfluoride: *a* — hematoxylin-eosin staining, ×100: *1* — PVDF fiber; *2* — PP Std fiber; *3* — connective tissue; *4* — neoperitoneum; *5* — neoperitoneum fenestration zone; *6* — muscles; *b* — hematoxylin-eosin staining, ×200: *1* — a mesh fiber; *2* — connective tissue; *3* — a vessel; *4* — neoperitoneum

the threads were exposed, having neither collagen nor mesothelial covering (Fig. 4, *a*).

The implantation zone surface of composite endoprostheses PVDF/R, PP Std/R, as well as PP Std/PVDF did not differ fundamentally and looked like continuously formed neoperitoneum. There was observed a continuous mesothelial layer, its thickness being the same throughout the preparation section. The entire surface had no fenestration zones (Fig. 4, *b*). The amount of collagen and vessels in the knitted structure zone of the mesh depended on its composition, the same as described above. In PVDF/R implantation, there was no inflammatory component. Mesh fibers were surrounded by delicate connective tissue and adequately integrated in abdominal wall tissues. The morphological findings were as follows.

Post-implantation time	The thickness of connective tissue capsule around endoprosthetic elements, μm								
	PP Std			PVDF			R		
	Me	Q1	Q3	Me	Q1	Q3	Me	Q1	Q2
30 days	110.5	49.25	194	56	34.25	78.75	24.5	20	28.75

The connective tissue formation around the mesh elements had its unique features. Maximum thickness of a capsule was found after PP Std implantation, smaller thickness — in PVDF endoprosthesis repair, minimum — in R mesh repair. In all cases the differences in capsule thickness were statistically significant: PP Std and PVDF compared: p=0.0469; Z=0.191; PVDF and R compared: p=0.0008; Z=0.027; PP Std and R compared: p=0.0000007; Z=0.393.

Discussion. Within the frame of the present study the authors did not set the task to prove which endoprosthesis is better (this approach would not comply with the generally accepted policy in world herniological practice). Nevertheless, a number of distinctions we found in the course of the reparative process due to mesh material and its structure is to be taken into consideration when choosing an endoprosthesis and its implantation technique.

PP Std mesh maximally induces connective tissue formation around its elements. This factor seems to be important in case of high risk of hernia recurrence (hernias, numerous recurrences and reoperations, obesity, etc). The use of PP Std in this situation will enable to reduce possible recurrences. But this mesh is to be placed as far as possible from abdominal organs not to induce excessive collagenogenesis in the abdominal cavity and avoid the development of a coarse adhesive process. Preperitoneal and intraperitoneal positions are not optimal, while onlay technique in this case is quite reasonable.

R implant results in minimum connective tissue formation. It is not indicated in high risk of recurrent hernias. On the other hand, the mentioned results can be considered positive in intraperitoneal position of endoprostheses, since the risk of coarse adhesions between the zone of prosthetic repair and the abdominal organs is to be estimated as minimum. If a mesh is required to be removed, the procedure will be relatively easily performed. This characteristic will be of advantage, if R endoprosthesis is used for temporary abdominal cavity closure (laparotomy in widespread peritonitis).

PVDF endoprosthesis induces the formation of a connective tissue capsule, the thickness of which should be considered to be satisfactory but not excessive. The range of implantation potential of this mesh appears to be the widest.

The findings agree with the data of experimental studies with a detailed macroscopic analysis [13, 18, 19]. They are consistent with the data received in clinical and morphological studies [17, 20]. However, some common factors demonstrated in this work were revealed for the first time.

A reparative process with knitted endoprostheses is accompanied by connective tissue extension through a mesh structure on the abdominal wall side. In minimal peritoneal alteration an adhesive process is minimal, but neoperitoneum structure is imperfect. Otherwise, excessive collagenogenesis develops, and a fixed intestinal wall has inflammation, with no neoperitoneum formation. These phenomena condition adhesions and fistulas. The mentioned phenomena are characteristic of PP Std, but can occur when other materials are used. PVDF fibers do not create conditions for marked taxis of inflammatory pool cells, and after repair fibroblast migration remains adequate, all the conditions necessary for connective tissue formation are present. However, PVDF induces collagen synthesis to a lesser extent, its amount being always sufficient for mesh integration in abdominal wall tissues but not excessive. The reported property is preferable for IPOM, since it has been proved before that the presence of a large amount of collagen on the implantation zone surface can be associated with an adhesive process on the side of abdominal cavity [13]. The combination of two components by weaving (PP Std/PVDF) improves the results, and in some cases is acceptable, though neoperitoneum is fenestrated more frequently above PP Std fibers. When a composite mesh with complete adhesive covering on visceral side is used, a reparative process results in complete neoperitoneum formation on visceral surface of the entire implantation zone. The composition of woven mesh component causes different structure of the forming connective tissue, but mesothelization of anti-adhesive plate made of R component is always adequate.

The findings agree with the results obtained and reported by other authors [21].

The analysis of morphological picture of the experimental prosthetic repair zone in accordance with IPOM technique showed the following: any of the materials studied can be used for abdominal wall plasty, though having its advantages and disadvantages. The use of PP Std mesh is associated with maximum collagenogenesis and its well integration in abdominal wall tissues, the presence of inflammation and adhesive process with the hollow organs involved. It should be expected that in clinical conditions intraperitoneal repair by the mentioned material can be accompanied by prolonged local inflammatory response, an adhesive process, but the number of recurrences will be minimal.

PVDF usage is preferable in terms of complication prevention. These data are consistent with the results of the previous experimental and clinical studies [17, 20].

The application of R mesh is the only possible and reasonable for temporal closure of the abdominal cavity, especially under the conditions of the pre-existing inflammation. An endoprosthesis will provide mesothelization and anti-adhesive effect, but will not induce inflammation and collagenogenesis, and if necessary, can be easily removed. Such situation is frequent in surgical practice when managing the patients with widespread peritonitis by an open operation. The experimental findings are consistent with the first clinical experience of using R endoprostheses as special polymer covering for laparotomy [22].

The abdominal wall closure by IPOM technique presupposes the combination of anti-adhesive properties of the endoprosthesis visceral surface and the firm integration of the mesh in abdominal wall tissue. In the course of the morphological study we found these characteristics to be typical of composite meshes investigated in this study. The obtained data absolutely agree with earlier macroscopic analysis of implantation zones [19]. Therefore, the application of the mentioned implants should be recognized as a safe technology concept by taking into consideration the morphology and pathologic physiology of a reparative process.

Conclusion. A reparative process course depends on mesh material and structure that should be taken into consideration when choosing a technique for their implantation. The use of standard polypropylene for intraperitoneal plasty without isolation procedure is possible in exceptional cases. PVDF implants have a number of advantages in intraperitoneal implantation and can be extensively used. Reperen endoprostheses are appropriate for temporary closure of the abdominal cavity best of all. The use of composite synthetic meshes with fibrous (knitted) structure of parietal side and complete adhesive cover on visceral side for intraperitoneal abdominal wall plasty is an optimal and morphologically proved approach.

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