ATJ, 2024, №2, 155-161

AZƏRBAYCAN TİBB JURNALI
AZERBAIJAN MEDICAL JOURNAL

EKSPERİMENTAL ŞƏRAİTDƏ MÜXTƏLİF İMPLANT TİPLƏRİNİN QARIN DİVARI TOXUMALARINA TƏSİRİ

V.P.Krışen[1], A.V.Kudryavtsev[2], N.N.Nor[1]
[1]Dnepr Dövlət Tibb Universiteti, Dnepr, Ukrayna
[2]Dnepr Dövlət Tibb Universitetinin əlahiddə "Universitet klinikası" struktur bölməsi, Dnepr, Ukrayna

Xülasə. *Periton toxumasının və qarın divarının əzələ-aponevroz qatının tirotrisin antibiotiki hopdurulmuş polipropilen implanta reaksiyasını öyrənmək məqsədilə tədqiqat aparılmışdır. Eksperimental tədqiqat 2022-2023-cü illərdə Dnepr Tibb Universiteti klinikasının laboratoriyasında kütləsi 200-240 q olan Wistar xəttinə mənsub erkən siçovullar hər birində 5 baş olmaqla 4 qrupa bölünmüşdür.*

Eksperimental tədqiqatın gedişində qarın divarının əzələ-aponevroz qatının və peritonun polipropilen tərkibli torla qarşılıqlı surətdə bir-birinə inteqrasiya etdiyi aşkara çıxmışdır. Bu zaman toxumaların implantla inteqrasiya etdiyi zonalarda aktiv surətdə kollagenogenez prosesləri və birləşdirici toxumanın aydın şəkildə damarlarla zənginləşməsi müşahidə edilmişdir. Tirotrisin antibiotiki hopdurulmuş torların ətrafında yaranan birləşdirici toxumanın morfoloji xüsusiyyətləri əhəmiyyətli dərəcədə fərqli olmuşdur ki, bu da orqanizmin toxumalarının bioloji cəhətdən polipropilen implantları ilə yaxşı uyğunlaşa bildiyini göstərir.

Açar sözlər: *qasıq yırtıqları, plastika, implantlar, qarnın ön divarı, birləşdirici toxuma*

Ключевые слова: *паховые грыжи, пластика, импланты, передняя брюшная стенка, соединительная ткань, коллагеногенез*

Key words: *inguinal hernia, plastic surgery, implants, anterior abdominal wall, connective tissue, collagenogenesis*

EFFECTS OF VARIOUS IMPLANT TYPES ON ABDOMINAL WALL TISSUE: AN EXPERIMENTAL STUDY

V.P.Kryshen[1], A.V.Kudriavtsev[2], N.N.Nor[1]
[1] Dnipro State Medical University, Dnieper, Ukraine
[2] Separate structural unit "University Clinic" of Dnipro State Medical University, Dnipro, Ukraine

The research studied the reaction of peritoneal tissue and the muscular aponeurotic layer of the anterior abdominal wall to a polypropylene implant treated with the antibiotic tyrothricin. The studies were conducted in the laboratory of the Dnieper Medical University clinic during 2022-2023. on 20 mature male Wistar rats weighing 200-240 g. The rats were divided into 4 groups of 5 animals each. An experimental study revealed the integration of tissue elements of the peritoneal walls and the muscular aponeurotic layer of the abdominal wall with the material of polypropylene mesh. At the same time, active processes of collagenogenesis occurred in the border zones and the area of integration with the implant, as well as pronounced manifestations of vascularization of the connective tissue. The morphological features of the connective tissue formed in the area and around intact implants and meshes treated with tyrothricin did not differ significantly from each other, which indicates the positive biocompatibility of body tissues and polypropylene implants.

Introduction. Surgical treatment of hernias using polypropylene implants, according to many authors, ensures the elimination of tissue defects, stabilization of the abdominal wall and guarantees its long-term durability [1,2]. In recent years, scientific efforts have been focused on modifying the surface of implants. Simultaneously with the creation of polypropylene meshes, implants from foamed polytetrafluoroethylene, arboxymethylcellu-

lose, polyethylene, polyglycolic acid, polymers of organic acids and materials based on various nanoparticles and hydrogels were developed and studied. The main requirement for these materials is the possibility of their integration with body tissues as a result of the development of connective tissue around foreign materials [3]. Despite a large number of attempts, the most optimal option for developing mesh implants turned out to be polypropylene material. It has been and remains a major advance in hernia surgery due to a number of properties, such as increased strength, flexibility, satisfactory tissue response and better biocompatibility compared to other materials [4,5,6]. When treating complicated hernias, one of the main problems is the development of severe infectious complications, which significantly worsen the effectiveness of therapy and the prognosis of the disease. In this regard, implants after coating with antimicrobial pharmacological agents began to be developed and introduced into surgical practice. [7,8]. The results of clinical observations indicate the high antimicrobial properties of antiseptic biocomposite meshes in patients. These meshes block the development of postoperative purulent complications. At the same time, the risks of developing bacterial contamination of tissues after surgery are reduced [9, 10]. The study is devoted to studying the effectiveness of using various types of meshes (without and with the use of an antimicrobial agent), their biocompatibility with body tissues and the reaction of peritoneal connective tissue under experimental conditions.

Purpose of the study. To study the reaction of the peritoneum to polypropylene implants treated with the antimicrobial agent tyrothricin.

Materials and methods: The studies were carried out in the laboratory of the Dnieper Medical University clinic during 2022-2023 on 20 mature male Wistar rats weighing 200-240 g. The rats were divided into 4 groups of 5 animals each. The first group (CGr) is the control group (n=5). The second (Gr-2) comparison group (n=5), which underwent surgery: an incision in the hypogastric region along the midline up to 1 cm long. In this case, the skin was dissected, the aponeurosis of the rectus abdominis muscles to the peritoneum. It was separated from the inner sheet of the muscle aponeurosis without damaging it. Later, the specified incision of all layers of the abdomen was sutured layer by layer with 7-0 vicryl thread (Ethicon, USA). The third (Gr-3) is the main group (1), (n=5). Here, a Covidien Parietene polypropylene implant measuring 1.0×0.5 cm (Medtronic plc, USA) was placed between the peritoneum and the inner layer of the aponeurosis of the rectus abdominis muscles without additional fixation. In animals of the fourth main group (Gr-4), (n=5), a similar implant was used, additionally treated with tyrothricin (Tyrosur, Engelhard Arzneimittel GmbH & Co. KG, Germany). The animals were operated on under thiopental sodium anesthesia with a 1% solution of the drug (single dose, 60 mg/kg, intraperitoneally) (PJSC Kievmedpreparat, Ukraine). The rats were fixed in a supine position, and an abdominal wall defect was simulated to place a polypropylene implant for 2 weeks. On the 14th day of the experiment, the animals were slaughtered after administering a lethal dose of sodium thiopental solution. The object of the study was the isolation of the peritoneal wall in animals of all studied groups. These tissues were fixed in a 10% formaldehyde solution prepared in phosphate buffer (pH 7.4). At the same time, the period of fixation of samples of seized tissue during the slaughter of animals was 24 hours. The latter were embedded in paraffin (Leica Surgipath Paraplast Regular, Germany) using the isopropanol-paraffin method. Paraffin sections 6 μm thick were prepared using a Thermo Microm HM 360 microtome (Thermo Scientific, USA) and placed on prepared glass slides (Sail Brand, China). After deparaffinization, histological sections were stained with hematoxylin and eosin. The next stage of the work was dehydration and enclosing the stained tissue sections under a cover glass (Sail Brand, China) in balsam (Merck, Germany). Histological sections were examined to study the development of structural changes in the peritoneum of control and experimental animals. To further study the collagen density, dewaxed sections were stained with another dye, Direct Red 80 (Magnacol Ltd, UK) for 20 min at 25°C [11]. Stained sections were also dehydrated and embedded in balm (Merck, Germany). Further histological examination of micropreparations was carried out after taking tissue sections using an Olympus BX51 microscope (Olympus, Japan) at a magnification of ×200. The peculiarity of the work was that this area of tissue was in direct contact with the mesh implant. In this area of the peritoneum, the thickness (μm) of connective tissue was measured, as well as in the musculoaponeurotic area using Carl Zeiss software

(AxioVision SE64 Rel.4.9.1, Germany). The specific density (%) of peritoneal collagen, at the level of its interaction with the mesh, was measured using ImageJ software (Wayne Rasband, USA) (algorithm: RGB image conversion to 8-bit, "threshold", correction of maximum PSR values positive region and dimension).

Statistical processing of the obtained data was carried out using the Origin v.9.0 program (OriginLab, USA). The significance of differences between comparison groups was determined using the Student method. Differences between groups were considered statistically significant at $P<0.05$. All data are presented using random effects to calculate the difference between the mean and standard deviation (M±m).

Absolutely all manipulations with animals were carried out in accordance with the requirements of the European Convention for the Protection of Vertebrate Animals, which are used for experimental and other purposes (Strasbourg, 1986) and the Law of Ukraine No. 3447-IV, Art. 26 "Rules for the treatment of animals used in scientific experiments, testing, educational process, production of biological products" dated 21.02.2006.

Results and its discussion. A study was carried out of the area of the peritoneum that was in contact with polypropylene implants. The latter were deposited in the abdominal cavity of animals of the Gr-3 and Gr-4 groups. Only a thin layer of fibrous connective tissue was found in the peritoneal wall of the CG. Individual blood vessels, namely small arterioles and venules, were identified between the connective and muscle tissues. In the midline of the abdomen, a thin layer of connective tissue was visualized, devoid of muscle elements with capillaries (Fig. 1).

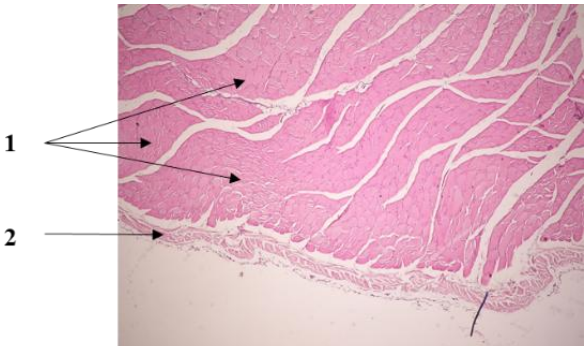

Fig. 1. *Peritoneal wall of intact rats (CG). 1 – muscle tissue; 2 – aponeurosis. Hematoxylin-eosin staining. Magnification ×200.*

In Gr-2 animals that underwent surgery without mesh deposition, dystrophic altered muscle fibers and the formation of connective tissue containing collagen were detected; small arteries and veins were visualized in the newly formed connective tissue, in the lumen of which there was blood stasis without obvious signs of the development of an inflammatory reaction. A heterogeneous pattern of connective tissue and its heterogeneous density were observed (Fig. 2).

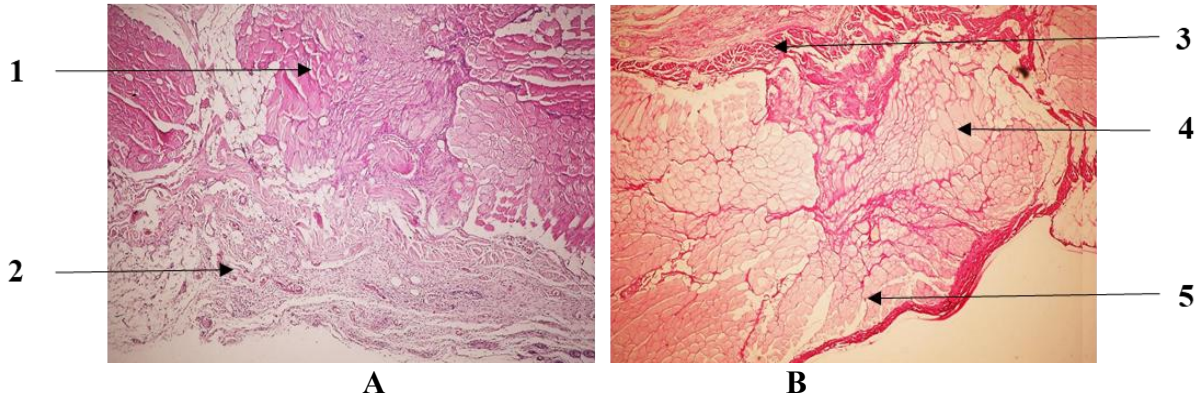

Fig. 2. *Area of defect in the wall of the hypogastric region (Gr-2).*
A *– damage to the rectus abdominis muscles and aponeurosis, development and thickening of the connective tissue layer. Hematoxylin-eosin staining. Magnification ×200.*
B *– development of fibrosis in the area of muscle and peritoneum atrophy. Stained with red serius. Magnification ×200.*
1 – damage to muscle tissue; 2 – connective tissue hyperplasia; 3 – striped muscle tissue; 4 – connective tissue hypertrophy; 5 – fibrosis phenomenon.

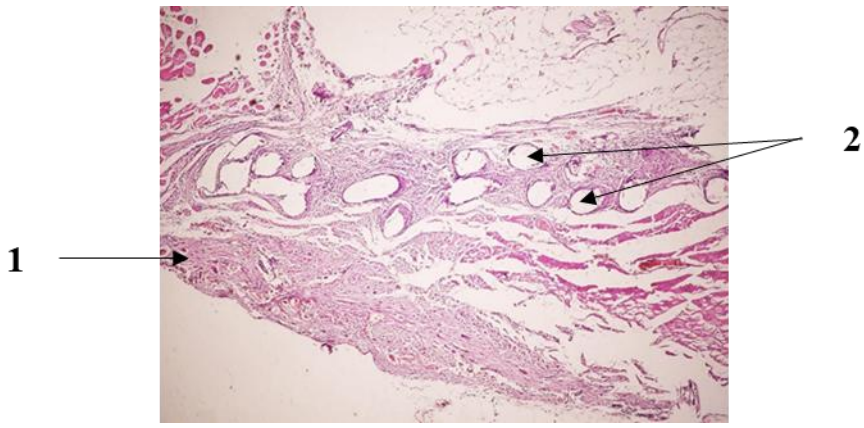

Fig. 3. *Site of implantation of polypropylene mesh (Gr-3).*
1 – connective tissue hypertrophy (collagenogenesis); 2 – grid section.
Hematoxylin-eosin staining. Magnification ×200.

In research animals Gr-3, after deposition of the polypropylene implant into the peritoneum of the hypogastric region, a slightly different picture of histological changes was observed: connective tissue surrounded the implant along the entire perimeter of the mesh, which indicated partial integration of the exogenous synthetic material with the tissue elements of the muscular aponeurotic area and the peritoneum of the animals. Perifocal muscle tissue also underwent corresponding structural remodeling changes. There was a decrease in the density and diameter of muscle fibers and an increase in intercellular space in muscle tissue. In these areas, where muscle fiber atrophy was detected, neoangiogenesis processes occurred through the sprouting of capillaries, as well as small arterioles and venules (Fig. 3).

In Gr-4 animals, a polypropylene implant treated with tyrothricin was implanted in the muscular aponeurotic area. Connective tissue development was observed in the implant area. The comparative analysis data indicated that the histological features of connective fibrous tissue did not differ significantly from the similar picture that was visualized in Gr-3 animals. Phenomena of general integration of the antibiotic-treated implant with the peritoneal tissue were observed (Fig. 4).

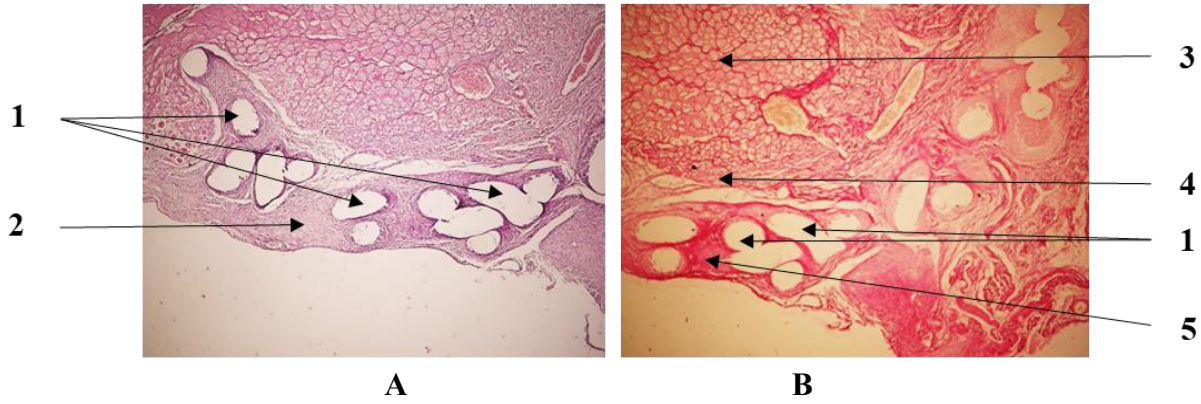

Fig. 4. *Interaction of tissues of experimental rats with a polypropylene mesh treated with tyrothricin (Gr-4)*
A *– development of dense fibrous tissue in the area where the implant is placed. Hematoxylin-eosin staining. Magnification ×200.*
B *– integration of muscular and peritoneal components with the implant. Collagen accumulation. Consolidation of the connective tissue layer in the area of the mesh treated with tyrothricin. Stained with red serius. Magnification ×200.*
1 – grid section; 2 – zone of damage with the development of connective tissue; 3 – collagenogenesis; 4 – connective tissue; 5 – muscle tissue.

In order to quantify the connective and fibrous tissue around the implant, a study of the thickness of the muscular aponeurotic region and densitometry of collagen in the connective tissue was carried out. Thus, in animals from the CG the peritoneal wall was undamaged, so no stained material was found for evaluation. At the same time, the thickness of the intact peritoneum in CG rats averaged 51,73±9,87 μm, and the main structural element of the intercellular matrix turned out to be collagen, the specific density of which was 7,84±0,42%. These results were used for the next and comparative stage of studying the damaged peritoneal wall during surgery or placement of polypropylene meshes in the abdominal cavity. In experimental Gr-2 rats, positively stained zones of collagen accumulation were observed in the peritoneum and partially damaged striated muscle tissue of the abdominal wall. In this case, the specific density of collagen was 12.68±5.15%. This indicates the activation of neocolagenogenesis processes in the extracellular matrix. During surgical intervention, polypropylene meshes placed in the abdominal cavity, other manifestations of the reaction of abdominal tissue to a foreign stimulus were recorded. In Gr-3 rats, an increased number of collagen fibers was observed along the perimeter of the implant. At the same time, collagen density increased significantly to 27.26±6.09% (P<0.05). At the same time, the volume of connective tissue in the muscular aponeurotic area also increased compared to similar indicators in animals of the CG and Gr-2 groups. The thickness of the connective tissue was 252.57±67.65 μm (p<0.05) (Table). Thus, body tissues react differently to the placement of meshes in the abdominal cavity to close a hernia defect, which should be taken into account when carrying out appropriate surgical interventions in patients with strangulated hernias.

In preparations from Gr-4 animals, a significant number of positively stained areas with an increased amount of collagen were found compared to CG and Gr-2. Collagen was concentrated not only around the polypropylene implant, but also along the entire contour of the polymer material and was found in certain areas of the striated muscles of the abdominal wall. In this case, the specific density of collagen was the same as in MG_1 animals, but slightly lower and amounted to 26.80±7.19% (p<0.05) (Table 1). The statistically significant results obtained are typical for animals Gr-3 and Gr-4 of the experimental groups, in which the specific density of collagen was higher than in animals CG and Gr-2. At the same time, these changes differed little from each other in animals Gr-3 and Gr-4 of the experimental groups in terms of the size of connective tissue and the specific density of collagen. As shown by the results of the studies, polypropylene implants of both types deposited in the abdominal cavity were partially integrated into the body tissues and, first of all, into the aponeurotic-muscular region of the rectus abdominal wall muscles and the peritoneum. At the same time, no pronounced manifestations of an acute inflammatory reaction to the implants were detected.

Table. Specific density of collagen and thickness of connective tissue of the peritoneum located around the implanted mesh in the hypogastric region of the abdominal cavity of experimental animals (M±m, n=20)

Group of animals	Conditions of experience	Tested indicators	
		Specific density of collagen (%)	Connective tissue thickness (мкм)
1	CG (*n=5)*	7,84±0,42	51,73±9,87
2	Gr-2 (*n=5)*	12,68±5,15	199,83±36,80*
3	Gr-3 (*n=5)*	27,26±6,09*	252,57±67,65*
4	Gr-4 (*n=5)*	26,80±7,19*	215,30±38,22*

*Note: * – probable difference compared to the control group (p<0.05).*

It is known that tissue reactions to the implantation of meshes made of different materials differ little from each other. This also applies to the formation of connective tissue, vascularization and the development of complications [12]. The end result of tissue reactions to the implant is the activation of connective tissue development. The first morphological signs of collagen formation around the implant under experimental conditions are observed already 3 days after surgery [13,14]. This aspect of the problem is of increased interest among specialists due to the fact that in laboratory animals (rats, mice) metabolic processes occur much more intensely than in humans. And at the same time, the formation of hypertrophic scar tissue, when the skin is damaged in laboratory rats, is recorded within 24 weeks, that is, for quite a long time [15]. But even after 6 months, the newly formed scar tissue does not reach the strength parameters of the fascia [16,17]. It should be noted that during our studies, which lasted for 14 days, the formation of connective tissue around the implant occurred. After 15-24 months, degradation of the implant and the concomitant development of inflammatory reactions in the tissues are possible. The formation of collagen around the mesh continues further with the continuation of the accumulation of its different types [18]. The results of our studies indicate an active reaction of connective tissue elements and the development of regenerative processes at the earliest stages of deposition of polypropylene mesh without coverage with tyrothricin. This may have not only scientific, but also important practical significance for hernia surgery, especially in the surgical treatment of patients with strangulated and infected hernias.

Conclusions

1. The processes of activation of fibroblasts, new formation of connective tissue against the background of pronounced vascularization contributed to the pronounced integration of peritoneal tissue and muscular aponeurotic areas of the anterior abdominal wall with the surface of mesh implants processing with tyrothricin.

2. The results of our research indicate the active regenerative development at the earliest stages of deposition of polypropylene meshes, which has not only scientific, but also important practical significance in the surgical treatment of patients with strangulated and infected hernias.

REFERENCES

1. Saiding Q., Chen Y., Wang J., et al. Abdominal wall hernia repair: from prosthetic meshes to smart materials // Mater Today Bio. 2023 Jun 29;21:100691. doi: 10.1016/j.mtbio.2023.100691
2. Liu Z., Wei N., Tang R. Functionalized strategies and mechanisms of the emerging mesh for abdominal wall repair and regeneration // ACS Biomater. Sci. Eng. 2021;7(6):2064-82. doi: 10.1021/acsbiomaterials.1c00118
3. Corduas F., Lamprou D.A., Mancuso E. Next-generation surgical meshes for drug delivery and tissue engineering applications: materials, design and emerging manufacturing technologies // Bio-des. Manuf. 2021;4;278-310.
4. Smith S.M., Khoja A.A., Jacobsen J.H.W, et al. Mesh versus non-mesh repair of groin hernias: a rapid review // ANZ J Surg. 2022 Oct;92(10):2492-99. doi: 10.1111/ans.17721
5. Yu S, Ma P. Mechanical properties of warp-knitted hernia repair mesh with various boundary conditions // J Mech Behav Biomed Mater. 2021 Feb;114:104192. doi: 10.1016/j.jmbbm.2020.104192.
6. Wilson R.B., Farooque Y. Risks and Prevention of Surgical Site Infection After Hernia Mesh Repair and the Predictive Utility of ACS-NSQIP // J Gastrointest Surg. 2022 Apr;26(4):950-64. doi: 10.1007/s11605-022-05248-6.
7. Mirel S., Pusta A., Moldovan M., Moldovan S. Antimicrobial Meshes for Hernia Repair: Current Progress and Perspectives // J Clin Med. 2022 Feb 8;11(3):883.
8. Pérez-Köhler B., García-Moreno F., Brune T. et al. Preclinical Bioassay of a Polypropylene Mesh for Hernia Repair Pretreated with Antibacterial Solutions of Chlorhexidine and Allicin: An In Vivo Study. PLoS ONE. 2015;10:e0142768.
9. Díez-Pascual A.M. Biopolymer Composites: Synthesis, Properties, and Applications // Int J Mol Sci. 2022 Feb 18;23(4):2257.
10. Haqverdiyev B.D. Improving the results of surgical treatment of hernias of the anterior abdominal wall in elderly patients // Azerbaijan Medical Journal, 2024, №1, p. 45-48. DOI/1034921/amj.2024.1.007
11. Wegner K.A., Keikhosravi A., Eliceiri K.W., Vezina C.M. Fluorescence of Picrosirius Red Multiplexed With Immunohistochemistry for the Quantitative Assessment of Collagen in Tissue Sections // J Histochem Cytochem.

2017 Aug;65(8):479-90.
12. Tanasescu C., Moisin A., Mihetiu A. et al. The use of polypropylene mesh in inguinal hernia surgery: A retrospective study // Exp Ther Med. 2021 Oct;22(4):11932.
13. Biondo-Simões Mde L., Morais C.G., Tocchio A.F. et al. Characteristics of the fibroplasia and collagen expression in the abdominal wall after implant of the polypropylene mesh and polypropylene/polyglecaprone mesh in rats // Acta Cir Bras. 2016 May;31(5):294-9.
14. Ribeiro W.G., Nascimento A.C.C, Ferreira L.B. et al. Analysis of tissue inflammatory response, fibroplasia, and foreign body reaction between the polyglactin suture of abdominal aponeurosis in rats and the intraperitoneal implant of polypropylene, polypropylene/polyglecaprone and polyester/porcine collagen meshes // Acta Cir Bras. 2021 Sep 3;36(7):e360706.
15. Zhou S., Wang W., Zhou S. et al. A Novel Model for Cutaneous Wound Healing and Scarring in the Rat // Plast Reconstr Surg. 2019 Feb;143(2):468-477.
16. Elango S., Perumalsamy S., Ramachandran K., Vadodaria K. Mesh materials and hernia repair // Biomedicine (Taipei). 2017 Sep; 7(3):16. doi: 10.1051/bmdcn/2017070316.
17. Jimi S., Saparov A., Koizumi S., et al. A novel mouse wound model for scar tissue formation in abdominal muscle wall // J Vet Med Sci. 2021 Dec 9;83(12):1933-1942.
18. Miserez M., Jairam A.P., Boersema G.S.A. et al. Resorbable Synthetic Meshes for Abdominal Wall Defects in Preclinical Setting: A Literature Review // J Surg Res. 2019 May; 237:67-75.// doi: 10.1016/j.jss.2018.11.054

ВЛИЯНИЕ РАЗЛИЧНЫХ ТИПОВ ИМПЛАНТОВ НА ТКАНИ БРЮШНОЙ СТЕНКИ В ЭКСПЕРИМЕНТЕ

В.П.Крышень[1], А.В. Кудрявцев[2], Н.Н.Нор[1]

[1.]Днепровский государственный медицинский университет, г. Днепр, Украина
[2.]Отдельное структурное подразделение "Университетская клиника" Днепровского государственного медицинского университета, г. Днепр, Украина

Резюме. Изучали реакцию тканей брюшины и мышечно-апоневротического слоя передней брюшной стенки на полипропиленовый имплант, обработанный антибиотиком тиротрицин. Исследования проводились в лаборатории клиники Днепровского медицинского университета на протяжении 2022-2023гг. на 20-ти половозрелых крысах-самцах линии Wistar массой 200-240 г. Крысы были разделены на 4 группы по 5 животных в каждой. При проведении экспериментального исследования была обнаружена интеграция тканевых элементов стенок брюшины и мышечно-апоневротического слоя брюшной стенки с материалом полипропиленовых сеток. При этом происходили активные процессы коллагеногенеза в граничных зонах и области интеграции с имплантом, а также выраженные проявления васкуляризации соединительной ткани. Морфологические особенности соединительной ткани, сформировавшейся в области и вокруг интактных имплантов и сеток, обработанных тиротрицином, существенно не отличались между собой, что свидетельствует о положительной биосовместимости тканей организма и полипропиленовых имплантов.

Autor for correspondence:
Nadezdha N.Nor, Dnipro State Medical University, Dnieper, Ukraine
E-mail: nadenor@i.ua