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Biointegration of barrier membranes with different microstructures in hernia repair: preliminary assessment of morphological aspects

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Abstract. Introduction. It is key to create barrier meshes to prevent adhesive disease and strengthen the anterior abdominal wall during hernioplasty. A promising material for it may be a polymer based on vinylidene fluoride and tetrafluoroethylene (VDF-TeFE). We aimed to do a preliminary assessment of morphological aspects of biointegrating VDF-TeFE meshes with various microstructures during hernioplasty, with the most effective one to be later improved for a full preclinical study.

Materials and methods. The research was performed on 40 Chinchilla rabbits divided into 4 equal groups: group 1 underwent laparotomy without mesh implantation, the animals from groups 2–4 received a VDF-TeFE mesh barrier having different microstructures.

Results. The 1.9-mm pore size (group 2) promoted the formation of dense connective tissue and the growth of adhesions between the organs, the mesh, and the anterior abdominal wall. Pores with a 0.9-mm diameter (group 3) prevented the formation of adhesions, but did not contribute to the strengthening of the anterior abdominal wall. The hybrid mesh structure (group 4) was the most effective because it prevented recurrence of anterior abdominal wall hernia and contributed to a decrease in the formation of adhesions between intestinal loops and the endoprosthesis.

Conclusion. The barrier meshes of a hybrid structure made from VDF-TeFE are promising in hernioplasty to prevent adhesions and hernia recurrence. Further work needs to improve the endoprosthesis by modifying it with drugs that prevent the formation of adhesions. However, this issue requires a more in-depth study.

Keywords: biointegration, biopolymers, chronic inflammation, adhesive disease, hernia repair, copolymer of tetrafluoroethylene with vinylidene fluoride (VDF-TeFE)

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Морфологические аспекты биоинтеграции барьерных мембран с различными вариантами микроструктуры для целей герниопластики

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Резюме. Введение. Для профилактики спаечной болезни и укрепления передней брюшной стенки при герниопластике актуально создание барьерных мембран. Перспективным материалом для этого может

стать полимер на основе сополимера винилиденфторида с тетрафторэтиленом (vinylidene fluoride and tetrafluoroethylene, VDF-TeFE). Целью исследования являлась предварительная оценка морфологических аспектов биоинтеграции барьерных мембран из VDF-TeFE при герниопластике с различными вариантами микроструктуры для дальнейшего усовершенствования наиболее эффективного образца и проведения полноценного доклинического исследования.

Материалы и методы. Исследование выполнено на 40 кроликах линии Шиншилла, разделенных на четыре равные группы: группе 1 проводили срединную лапаротомию без имплантации мембраны, животным из групп 2, 3 и 4 имплантировали мембрану из VDF-TeFE с различной микроструктурой.

Результаты. Размер пор 1,9 мм (группа 2) способствовал образованию грубой волокнистой соединительной ткани и росту спаек между органами, мембраной и передней брюшной стенкой. Поры диаметром 0,9 мм (группа 3) препятствовали появлению спаек, однако не содействовали укреплению передней брюшной стенки. Гибридная структура мембраны (группа 4) оказалась наиболее эффективной, так как предотвращала рецидив грыжи передней брюшной стенки и способствовала снижению образования спаек между петлями кишечника и эндопротезом.

Заключение. Использование барьерных мембран гибридной структуры из VDF-TeFE перспективно при герниопластике для профилактики спаечного процесса и рецидивов грыж. Кроме того, дальнейшим направлением совершенствования эндопротеза будет его модификация лекарственными средствами, профилактирующими образование спаек. При этом данный вопрос требует дальнейшего более глубокого изучения.

Ключевые слова: биоинтеграция, биополимеры, хроническое воспаление, спаечная болезнь, герниопластика, сополимер винилиденфторида с тетрафторэтиленом (VDF-TeFE)

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Introduction

The occurrence of adhesive disease following abdominal surgeries, including hernioplasty, represents a significant challenge in modern surgery [1] on a par with the reinforcement of the anterior abdominal wall to prevent relapses [2]. The development of adhesive disease is associated with both trauma during surgical procedure and the subsequent inflammatory response [3]. Adhesive disease can lead to complications and significantly affect the patients' quality of life [4]. Therefore, the search for effective preventive measures against adhesive disease is crucial. These strategies can be categorized into three groups: surgical (intraoperative reduction of peritoneal trauma), pharmacological (the use of anticoagulants to prevent peritoneal ischemia, fibrinolytic therapy, anti-inflammatory medications, and agents aimed at inhibiting collagenogenesis), and mechanical (creating a barrier between the injured surfaces during surgery) [5].

One promising approach in the mechanical prevention of adhesive disease is the development of biocompatible nonresorbable membranes which are preferable to the resorbable ones due to their durability, resistance to biodegradation, capacity to minimize inflammatory responses, and the role in reinforcing the anterior abdominal wall during hernioplasty, thereby reducing recurrence risks [6]. Unlike resorbable membranes, the nonresorbable ones maintain

structural integrity, prevent the ingrowth of fibrous tissue into surrounding organs, and do not release inflammatory-inducing degradation products. Nonresorbable membranes include those made from polypropylene, polyethylene glycol, polylactic acid, chitosan, and other polymers. However, their application in hernioplasty is limited due to a number of drawbacks [7].

A prospective polymer to produce barrier meshes is copolymer of vinylidene fluoride and tetrafluoroethylene (VDF-TeFE). The materials derived from this polymer exhibit mechanical strength, proven biocompatibility, and piezoelectric properties; maintain their physical properties over time; enhance the tissue regeneration; reduce the risk of postoperative scarring; and do not chemically react with biological fluids [8].

This polymer is utilized to produce various biomaterials that are actively employed in different surgical fields at the Center for Additive Technologies of the National Research Tomsk Polytechnic University [9]. 3D printing enables the manufacture of barrier meshes of necessary sizes and with specified microstructures, which will be used in hernioplasty effectively performing anti-adhesive functions and meeting the needs of individual patients.

The aim of this research was to describe morphological aspects of biointegration of VDF-TeFE barrier meshes with various microstructure configurations in hernioplasty

in order to further refine the most effective sample and conduct a comprehensive preclinical study.

Materials and methods

The study was conducted at Siberian State Medical University and approved by the local ethics committee (protocol No. 15-2 dated April 1, 2024). All procedures involving animals were carried out in accordance with the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

The research was designed as a comparative exploratory investigation of morphological aspects of biointegrating three samples of mesh polymer membranes for hernioplasty, each with different microstructures, pore diameters, and polymer fill percentages. The samples were designed and manufactured at the Shared Access Additive Technology Center of the National Research Tomsk Polytechnic University using an FDM printer (Picaso Designer X Pro, Picaso 3D, Russia). The filament ($\varnothing=1.75$ mm) for the samples was prepared from a copolymer of VDF-TeFE (Galopolymer, Russia) with a tetrafluoroethylene content of 9% mol.

The experiment involved 40 male Chinchilla rabbits (2500–3000 g, aged 5–6 months) that were divided into four equal groups with a block randomization method. Group 1 was composed of the animals that underwent sham surgery, i.e., a midline laparotomy without subsequent mesh implantation. Group 2 was given a mesh with a “honeycomb” microstructure, a polymer fill percentage of 40%, and a pore diameter of 1.9 ± 0.1 mm. Group 3 had a mesh with a “honeycomb” microstructure, a fill percentage of 60%, and a pore diameter of 0.9 ± 0.1 mm. Group 4 received a bilayer mesh with a “honeycomb” microstructure, where the side facing the intestine had a polymer fill percentage of 70% and a pore diameter of 0.9 ± 0.1 mm, while the side facing the abdominal wall had a polymer fill percentage of 30% and a pore diameter of 1.9 ± 0.1 mm. In groups 2–4, the meshes were implanted in the anterior abdominal wall.

The animals were provided by the Goldberg Research Institute of Pharmacology and Regenerative Medicine (Tomsk, Russia). Prior to the experiment, the rabbits underwent a two-week acclimation period in the vivarium. The animals were kept according to the sanitary and epidemiological rules 2.2.1.3218-14 “On Sanitary and Epidemiological Requirements for the Design, Equipment, and Maintenance of Experimental Biological Clinics (Vivariums),” approved by the Russian Federation Chief State Sanitary Doctor Resolution No. 51 of August 29, 2014.

All animals underwent sterile procedures after atropine sulfate premedication (Dalchimpharm, Russia) at a dosage of 0.2 ml/kg subcutaneously. Thirty minutes later, they were anesthetized with Zoletil-100 (Vibrac, France) at a dosage of 10 mg/kg, and then the surgical field was treated with a triple antiseptic.

Afterwards, we performed a midline laparotomy. The meshes were fixed using sterile atraumatic needles and absorbable sutures made of polyglycolic acid of a 4/0 size with knot sutures at mesh corners.

The removal criteria included general deterioration (including lethargy, apathy, food refusal, sleep disturbances, fever) or intra-experimental death. The removal from the experiment was supposed to be followed by euthanasia. No animals were excluded from the study.

During postoperative monitoring, we did a macroscopic assessment of changes in the anterior abdominal wall. The animals were removed from the experiment on day 21 in a CO₂ euthanasia chamber (ZOOONLAB, Germany). Having euthanized the subjects, we performed laparotomy, made macroscopic evaluation of adhesions (diameter, thickness, length, type, and presence of vascularization), and took macroscopic pictures.

We obtained tissue samples for histological analysis from the wound defect area, capturing tissue from the periphery (5 mm). The tissues were fixed in a 10% neutral formalin solution (Biovitrum, Russia), washed under tap water, dehydrated in increasing concentrations of ethanol and isopropanol (Biovitrum, Russia), and subsequently embedded in paraffin (Biovitrum, Russia). We prepared 5- μ m-thick histological sections using a Leica SM 2010R rotary microtome (Leica, Germany). The sections were stained with hematoxylin and eosin (ABRIS+, Russia) and by the Van Gieson method using picric acid fuchsin (Biovitrum, Russia).

Histological preparations were examined with a light microscope (Axioskop 40, Carl Zeiss AG, Oberkochen, Germany: objectives $\times 10$, $\times 40$, and $\times 90$, eyepieces $\times 10$). In each group, we studied 100 fields and quantified the specific area of dense fibrous connective tissue, loose fibrous connective tissue, the frequency of adipose tissue (%), and cellular infiltration (%). The last parameter was calculated as the ratio of the studied parameter area to the total visible area within the field of view. Moreover, we qualitatively assessed the condition of the microcirculatory vessels and the cellular composition of tissues.

We analyzed the images using AxioVision 4.8 image processing software (Carl Zeiss, Germany) and ImageJ 1.52u (National Institutes of Health, USA) and performed statistical analysis using Statistica 10.0 software (StatSoft Inc., USA). The Kolmogorov-Smirnov test was employed to assess the distribution characteristics of the variables, and descriptive and nonparametric statistical methods were utilized for data analysis. The parameters were described as median and quartiles, Me (Q1; Q3). The Kruskal–Wallis test was used to compare independent samples. The differences were considered statistically significant at a significance level of $p<0.05$.

Results

All animals resumed their normal activities the next day after surgery; they began to eat and drink and also demonstrated restored intestinal activity. The postoperative wound

in all animals healed by day 7 of the study. By the end of the experiment, the animals lost on average 500 (± 150) g.

On macroscopic examination on day 21, the animals in group 1 had adhesions at puncture sites. In group 2, autopsy revealed a significant adhesive process within the abdominal cavity, with connective tissue fibers infiltrating the mesh, whose parietal layer of the membrane was firmly adhered to the anterior abdominal wall. A loop of the colon, which was pneumatic and tense, was attached to the visceral surface of the mesh via densely vascularized

adhesions; its wall was yellowish (Fig. 1 A). Blunt dissection of the adhesions was unsuccessful.

Macroscopic examination of group 3 revealed that adhesions did not infiltrate the mesh on the parietal side; however, the endoprosthesis was adhered to the anterior abdominal wall peripherally, which is likely to be due to its uneven sharp edges. An adhered loop of the small intestine was visualized on the visceral surface of the mesh (Fig. 1 B), with the adhesions being loose, amenable to blunt dissection, and exhibiting sparse vascularization.

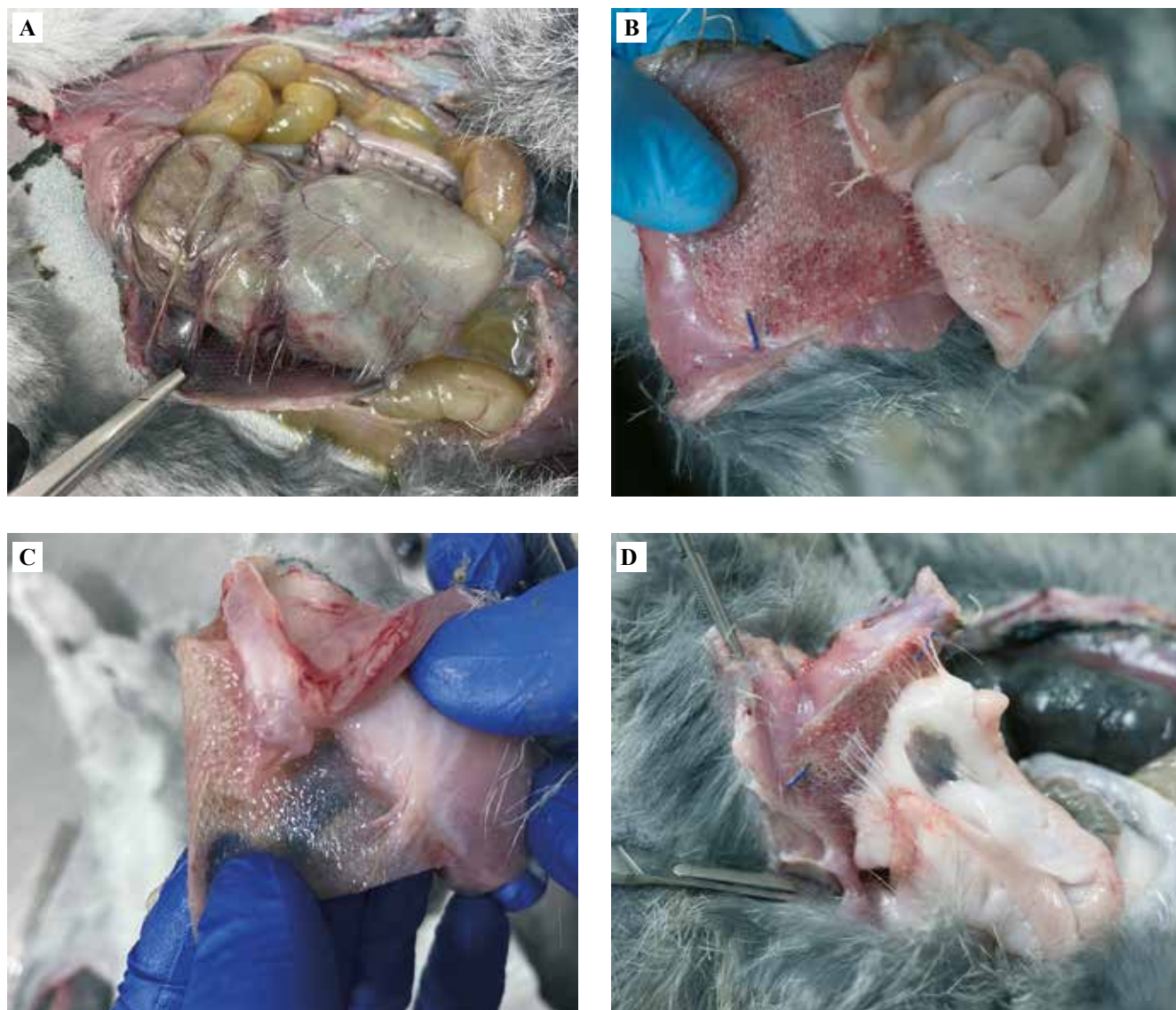


Fig. 1. Macroscopic view in the area of barrier membrane implantation in the anterior abdominal wall. Day 21.

A – a part of the colon adhered to the implant by numerous vascularized adhesions, group 2. B – the visceral surface of the endoprosthesis with a loop of the small intestine adhered to it by a thin adhesion, group 3. C – adhesions growing into the implant from the side of the anterior abdominal wall, group 4. D – spontaneous rupture of thin membranous adhesions when lifting the tissues of the anterior abdominal wall with tweezers, group 4

Рис. 1. Макроскопическая картина области имплантации барьерной мембраны в передней брюшной стенке. 21-й день.

А – участок толстой кишки, адгезированный многочисленными васкуляризованными спайками к импланту, группа 2. В – висцеральная поверхность эндопротеза с адгезированной к ней петлей тонкой кишки посредством слабовыраженной спайки, группа 3. С – прорастание спаек в имплант со стороны передней брюшной стенки, группа 4. D – механический разрыв тонких пленчатых адгезий при подъеме тканей передней брюшной стенки при помощи пинцета, группа 4

In group 4, macroscopic examination indicated that the parietal surface of the mesh was tightly fused with the tissues of the anterior abdominal wall (Fig. 1 C). A loop of the small intestine and its mesentery were adhered to the visceral surface via thin membranous adhesions; however, upon lifting the mesh with forceps, the adhesions spontaneously ruptured at a height of 1–2 cm (Fig. 1 D).

On day 21, the animals in group 2 exhibited an increase in dense fibrous connective tissue on both the visceral and parietal sides, with a specific area reaching 59.7% [53.1; 62.2] (Table). Additionally, there was loose fibrous connective tissue with a specific area that was 5.8 times significantly smaller compared to that of group 1 [$p=0.007$] (Table, Fig. 2 A). Among the connective tissue fibers, we observed small foci of adipose tissue, with a frequency of occurrence being 35.0% [33.1; 37.4] (Table, Fig. 2 B). We also detected extensive cellular infiltration, with a specific area 3.8 times greater compared to that of group 1 [$p=0.013$] (Table). Clusters of foreign-body giant cells and epithelioid cells were identified (Fig. 2 B). The formed connective tissue exhibited thin-walled, hyperemic blood vessels, as well as vessels showing signs of stasis and thrombosis (Fig. 2 C).

In group 3, there was a predominance of loose fibrous connective tissue and an increase in dense fibrous connective tissue at the periphery of the mesh (Fig. 3 A). Areas of dense fibrous connective tissue were found interspersed among the loose connective tissue. The specific area of dense fibrous connective tissue reached 34.3% (31.1; 39.1), which was 1.7 times lower compared to that of group 2 [$p=0.011$] (Table). Cellular infiltration was sparse, with

a specific area being 2 times smaller compared to that of group 2 [$p=0.015$] (Table). Foreign-body giant cells and epithelioid cells were observed in isolated fields of view, primarily at the periphery of the membrane. Adipose tissue associated with fibrosis was also identified at the periphery of the implant (Fig. 3 B), with a frequency of occurrence being 15.1% [11.5; 18.9] (Table). The vascularization of the newly formed vessels was minimal, and we did not observe signs of stasis, sludging, and thrombosis. This morphological characteristic of the implantation area was consistent for both the parietal and visceral sides relative to the mesh.

In group 4, with the hybrid mesh on the abdominal wall side, there was a predominance of poorly vascularized dense fibrous connective tissue with foci of cellular infiltration (Fig. 4 A). The specific area of dense fibrous connective tissue reached 76.2% (71.3; 78.9), which was 1.3 and 2.2 times greater than that in groups 2 and 3, respectively ($p=0.021$). On the side facing the abdominal organs, we visualized the predominance of loose fibrous connective tissue with a large number of blood vessels (Fig. 4 B, 4 C). Isolated foci of cellular infiltration were visualized; however, their specific area did not significantly differ from the values in group 1 ($p=0.083$). The specific area of dense fibrous connective tissue, which was arranged in separate longitudinally oriented bundles and corresponded to thin adhesions upon macroscopic examination, was 5.8 and 3.3 times smaller compared to that of groups 2 and 3, respectively [$p=0.035$] (Table, Fig. 4 D). We detected multinucleated giant cells and epithelioid cells in isolated fields of view and did not find adipose tissue associated with foci of fibrosis.

Table | Таблица

Morphological indicators of tissue changes in the area of mesh implantation in the anterior abdominal wall, Me (Q1; Q3) | Морфологические показатели изменения тканей в области имплантации барьерной мембраны в передней брюшной стенке, Ме (Q1; Q3)

	Group 1 Группа 1	Group 2 Группа 2	Group 3 Группа 3	Group 4 (visceral side / parietal side) Группа 4 (висцеральная сторона / париеальная сторона)
Specific area of loose connective tissue, % Удельная площадь рыхлой волокнистой соединительной ткани, %	93.7 (91.3; 95.6)##&	16.2 (12.5; 17.8)*&	53.6 (38.1; 45.7)*#	84.3 (79.1; 89.9)*##&/ 12.6 (8.1; 16.6)*&
Specific area of dense connective tissue, % Удельная площадь грубой волокнистой соединительной ткани, %	—	59.7 (53.1; 62.2)*&	34.3 (31.1; 39.1)*#	10.2 (7.1; 14.1)*##&/ 76.2 (71.3; 78.9)*##&
Specific area of cell infiltration, % Удельная площадь клеточной инфильтрации, %	6.3 (5.4; 8.1)	24.1 (22.6; 28.3)*&	12.1 (18.1; 29.1)*	5.5 (3.1; 7.6)##&/ 11.2 (9.6; 14.5)*#
Frequency of adipose tissue occurrence Частота встречаемости жировой ткани	—	35.0 (33.1; 37.4)*	15.1 (11.5; 18.9)*#	—#

* – significant differences compared to group 1 ($p<0.05$) | достоверные отличия с группой 1 ($p<0,05$)

– significant differences compared to group 2 ($p<0.05$) | достоверные отличия с группой 2 ($p<0,05$)

& – significant differences compared to group 3 ($p<0.05$) | достоверные отличия с группой 3 ($p<0,05$)

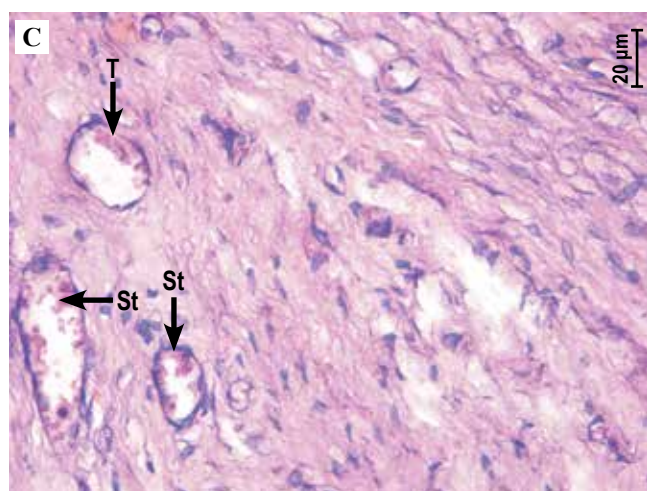
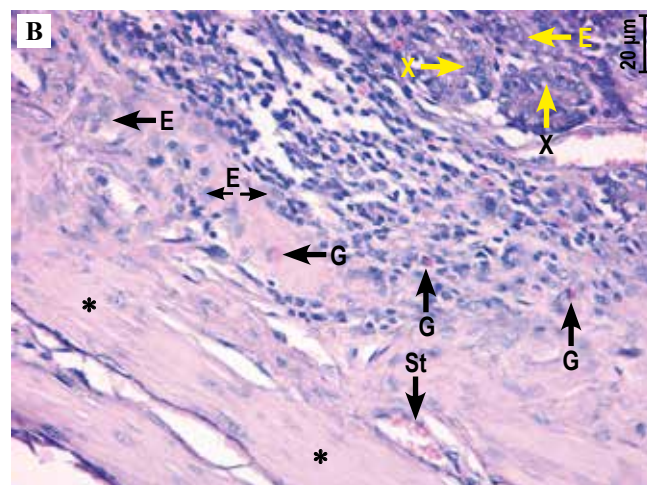
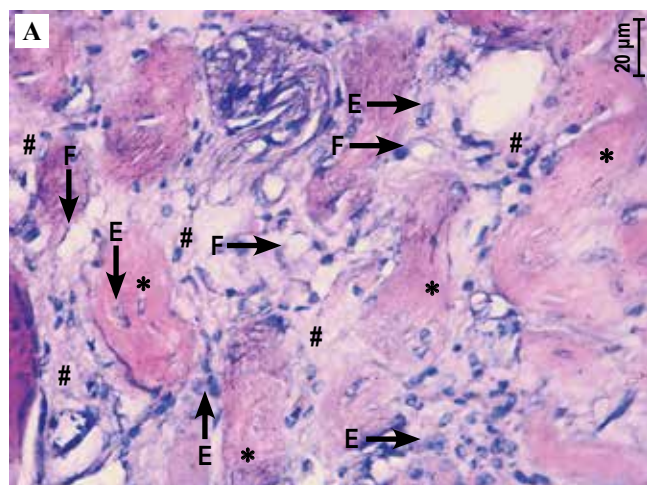


Fig. 2. Microscopic view in the area of barrier membrane implantation on day 21, group 2. H&E stain.

A, B, C – $\times 400$

* – dense connective tissue, # – loose connective tissue, F – adipose tissue, E – epithelioid cells, X – xanthomatous cells, G – foreign-body giant cells, St – stasis, V – venule, T – thrombosis

Рис. 2. Микроскопическая картина в области имплантации барьерной сетки на 21-е сутки исследования, группа 2.

Окраска гематоксилином и эозином. А, В, С – $\times 400$

* – грубоволокнистая соединительная ткань, # – рыхлая волокнистая соединительная ткань, F – жировая ткань, E – эпителиоидные клетки, X – ксантомные клетки, G – гигантские клетки инородных тел, St – стаз, V – венула, T – тромбоз

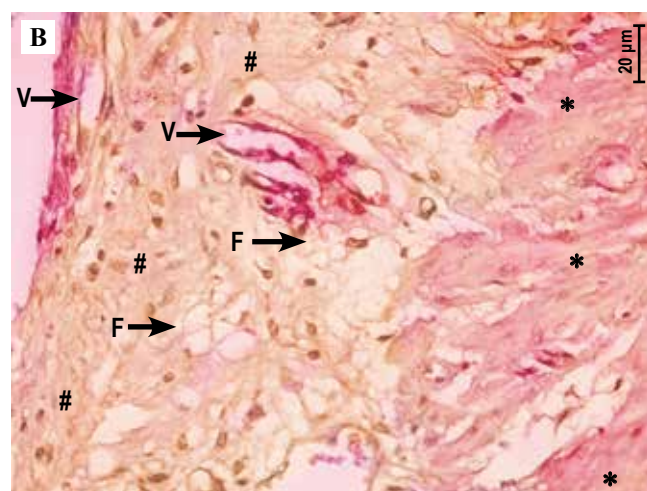
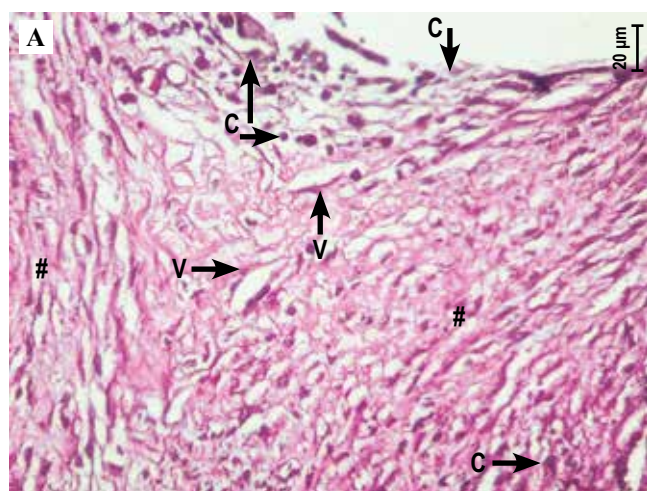


Fig. 3. Microscopic view in the area of barrier membrane implantation on day 21, group 3. Van Gieson's stain. A, B – $\times 400$

C – cell infiltration, V – venule, * – dense connective tissue, # – loose connective tissue, F – adipose tissue, E – epithelioid cells

Рис. 3. Микроскопическая картина в области имплантации барьерной сетки на 21-е сутки исследования, группа 3.

Окраска по ван Гизону. А, В – $\times 400$

С – клеточная инфильтрация, V – венула, # – рыхлая волокнистая соединительная ткань, F – жировая ткань, E – эпителиоидные клетки

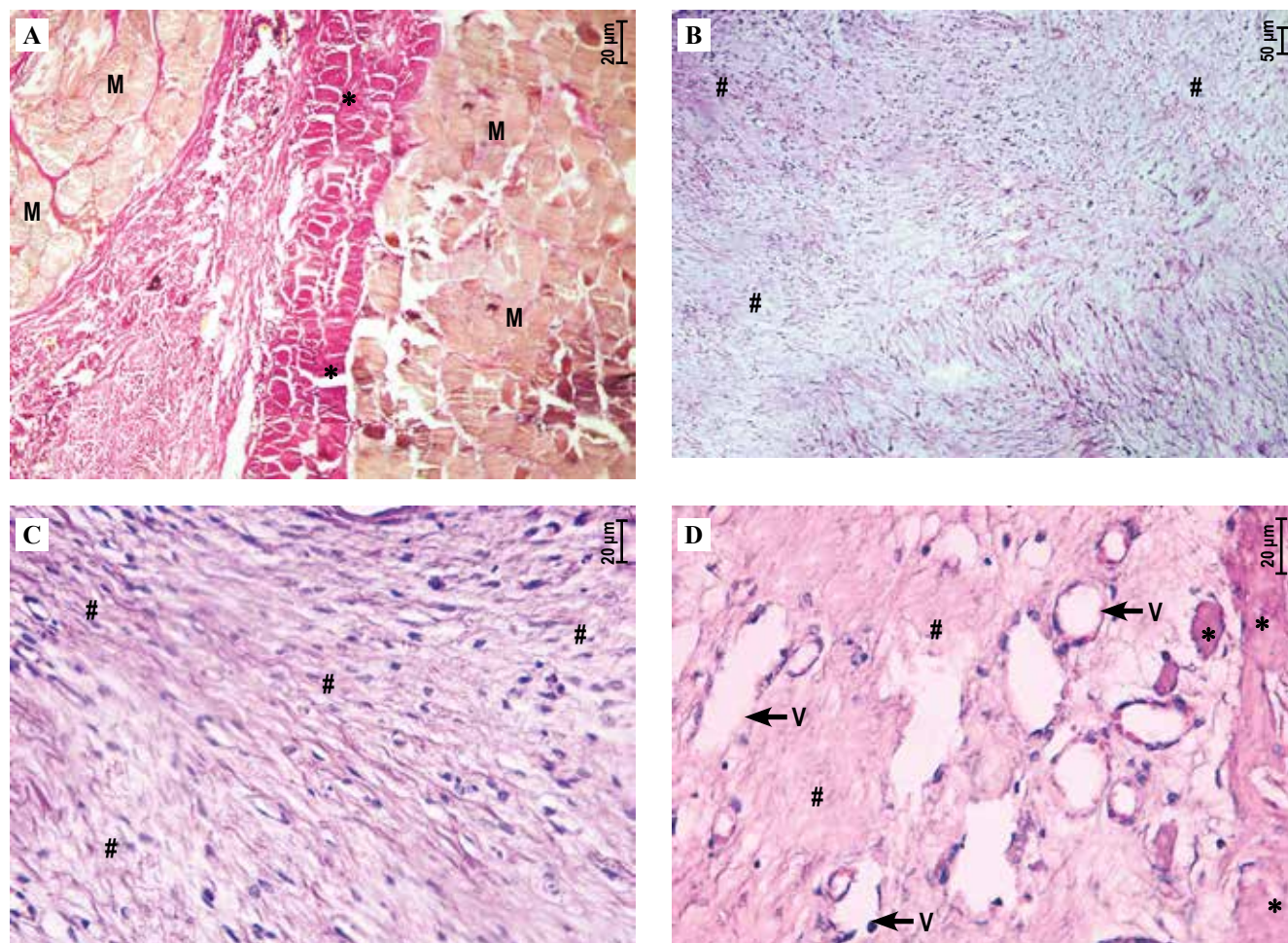


Fig. 4. Microscopic view in the area of barrier membrane implantation on day 21, group 4.

A – $\times 400$, Van Gieson's stain. B – $\times 100$, H&E stain. C, D – $\times 400$, H&E stain

* – dense connective tissue, M – muscle tissue, # – loose connective tissue, V – venule

Рис. 4. Микроскопическая картина в области имплантации барьерной сетки на 21-е сутки исследования, группа 4.

А – окраска по ван Гизону, $\times 400$, В – окраска гематоксилином и эозином, $\times 100$. С, D – окраска гематоксилином и эозином, $\times 400$

* – плотная соединительная ткань, М – мышечная ткань, грубоволокнистая соединительная ткань, # – рыхлая волокнистая соединительная ткань, V – венула

Discussion

Based on our literature analysis, we may conclude that adhesive disease can lead to numerous complications following hernioplasty [10]. There is a notable dissatisfaction among surgeons regarding the emerging interest of researchers in modifying the Lichtenstein hernioplasty technique. Consequently, the laparoscopic technique known as IPOM (Laparoscopic intraperitoneal onlay mesh) has been recognized as the safest and the most effective approach, which entails the intraperitoneal implantation of a mesh that completely covers the defect [11]. In this context, the development and implementation of effective barrier membranes represent a critical task in contemporary surgery [12].

In this study, we evaluated the preliminary results of using polymeric barrier meshes made from VDF-TeFE with varying microstructures for reinforcing the anterior

abdominal wall and separating organs to prevent adhesive process during hernioplasty.

The use of samples with the largest cell diameter (1.9 mm) in group 2 yielded negative results. We observed extensive ingrowth of richly vascularized adhesions into the mesh and surrounding tissues at the macroscopic level. At the microscopic level, we noted the formation of dense fibrous connective tissue underlying these adhesions on both the parietal and visceral sides.

Conversely, a different scenario was observed in experimental group 3, where the pore diameter was the smallest (0.9 mm). The adhesions were primarily located at the periphery of the mesh, particularly at its edges and suture sites, suggesting the need for modifications to the prosthesis—specifically, the creation of rounded corners and streamlined edges. The adhesions at the macroscopic level appeared whitish, and at the microscopic level, they were

poorly vascularized and predominantly consisted of loose fibrous connective tissue. Thus, we can conclude that a smaller pore diameter prevents the development of adhesions; however, it does not contribute to the reinforcement of the anterior abdominal wall.

Based on the data obtained from the morphological study of tissues in animals from groups 2 and 3, we decided to create a hybrid mesh consisting of a visceral layer with a 0.9-mm cell diameter and a parietal layer with a 1.9-mm cell diameter. This modification aimed to prevent the formation of adhesions between the abdominal organs and secure the membrane to the anterior abdominal wall through the formation of dense fibrous connective tissue. Preliminary morphological investigations indicated that this barrier mesh design aligned with the goals of surgical intervention in hernioplasty.

To further enhance the methods for preventing adhesive disease, it is essential to continue research aimed at the development and optimization of biocompatible materials, improving their physicochemical and biological properties [13]. Pharmacologically active coatings for nonresorbable meshes present new opportunities in preventing adhesive disease. Pharmacological classes of drugs that may potentially be applied in combination with nonresorbable materials include glucocorticoids, nonsteroidal anti-inflammatory drugs, inhibitors of proinflammatory cytokines, statins, antifibrinolytic agents, and antibacterial medications [14, 15].

Thus, this exploratory study has identified the primary characteristics of the barrier mesh, i.e., a hybrid two-layer structure that allows for integration into the anterior abdominal wall, thereby reinforcing it and preventing the formation of adhesions with the gastrointestinal organs and featuring streamlined edges and rounded corners. In future studies, we aim to modify the mesh surfaces with pharmacological agents.

Further comprehensive morphological research will focus on the comparative study of barrier meshes with properties validated during this investigation. The number of control points will be increased in accordance with the stages of tissue regeneration, i.e., alteration, exudation, and proliferation. The comprehensive morphological study will include an increased number of staining techniques for light microscopy, immunohistochemical analysis, and electron microscopy, which will facilitate the examination of all aspects of connective tissue formation, its vascularization and innervation, as well as the release of biologically active substances.

Conclusion

Based on our morphometric analysis, it can be concluded that the hybrid structure of the barrier mesh is preferable due to the following reasons. Firstly, it provides reinforcement of the anterior abdominal wall, which is a crucial aspect in preventing recurrences of anterior abdominal wall hernias. Secondly, it contributes to the reduction in adhesion formation between intestinal loops and the mesh.

Furthermore, we believe that it is promising to enhance the endoprosthesis by modifying its visceral surface with pharmacological agents that inhibit adhesion formation. However, this issue necessitates further in-depth investigation.

Author contributions

Conceived the study and designed the experiment – A.D. Koniaeva, E.Yu. Varakuta, A.V. Gerasimov, A.V. Potapov, E.N. Bolbasov. Collected the data and performed the analysis – A.E. Leiman. M.V. Fedosova, A.O. Vorobyev, U.V. Chernova. Wrote the paper – A.D. Koniaeva, E.Yu. Varakuta, A.V. Gerasimov, A.V. Potapov, U.V. Chernova. Edited the manuscript – E.Yu. Varakuta, E.N. Bolbasov.

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