EXPERIMENTAL RESEARCHES

FEATURES OF THE INTEGRATION OF TWO-LAYER METAL KNITWEAR MADE OF TITANIUM NICKELIDE DURING THE REPLACEMENT OF A THORACOABDOMINAL DEFECT IN THE EXPERIMENT

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ABSTRACT

The aim *of investigation was to study experimentally the morphological features of tissue integration of two-layer titanium nickelide (TiNi) knitwear when replacing thoracoabdominal defects.*

Materials and methods. *The experiments were carried out on 40 Wistar rats. The experimental animals were divided into two comparison groups: in Group A (n = 20) the defect was replaced using a two-layer knitted tape made of TiNi, in Group B (n = 20) a polypropylene mesh implant was used. The technique of the operation and the peculiarities of keeping the animals did not differ. Animals were taken out after 14, 30, 60 and 90 days of experiment. The macroscopic structural features at the site of implant fixation to tissues and at the sites of contact with underlying organs were studied, and the inflammatory process was assessed. The histological and electron microscopic study was carried out with an assessment of the features of tissue integration through the mesh structure of knitwear.*

Results. *Thirty days after the surgery in four cases of Group B the appearance of the chest wall deformation at the site of implant fixation was noted, in one case the deformation site was located along the lateral edge of the abdominal wall. Among the animals of Group A no such changes were recorded. The histological and electron microscopy examination revealed that the porous structure of the TiNi wire, as well as the biomechanical and biochemical properties of the two-layer metal knitwear, ensure optimal integration of the endoprosthesis in the body tissues, forming an elastic frame close to natural. In Group B, on the contrary, the reaction of the body caused by the implanted polypropylene prosthesis was characterized by more pronounced fibrosis, and tissue integration through the mesh structure of the implant was not observed.*

Conclusion. *Two-layer TiNi knitwear in the replacement of complex structures of the thoracoabdominal zone showed promising results, which opens up prospects for further clinical research.*

Key words: *two-layer knitwear, titanium nickelide, mesh implant, replacement of post-resection defects, tissue integration, biocomparability*

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ОСОБЕННОСТИ ИНТЕГРАЦИИ ДВУХСЛОЙНОГО МЕТАЛЛОТРИКОТАЖА ИЗ НИКЕЛИДА ТИТАНА ПРИ ЗАМЕЩЕНИИ ТОРАКОАБДОМИНАЛЬНОГО ДЕФЕКТА В ЭКСПЕРИМЕНТЕ

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РЕЗЮМЕ

Цель исследования. *Изучить в эксперименте морфологические особенности тканевой интеграции двухслойного металлотрикотажа из никелида титана (TiNi) при замещении торакоабдоминальных дефектов.*

Материалы и методы. *Проведена серия экспериментов на 40 крысах линии Wistar. Экспериментальные животные были разделены на две группы сравнения: в группе А (n = 20) замещение дефекта осуществляли с применением двухслойной трикотажной ленты из TiNi; в группе В (n = 20) использовался сетчатый имплант из полипропилена. Техника операции и особенности содержания у животных в группах не отличались. Животных выводили из эксперимента через 14, 30, 60 и 90 суток после операции. Изучали макроскопические структурные особенности в месте фиксации имплантата к тканям и на участках контакта с подлежащими органами, оценивали воспалительный процесс. Проводили гистологическое и электронно-микроскопическое исследование с оценкой особенностей интеграции тканей сквозь сетчатую структуру металлотрикотажа.*

Результаты. *В ходе роста животных и набора веса через 30 суток в четырёх случаях в группе В отмечено появление деформации в месте фиксации импланта к грудной стенке, в одном случае участок деформации был расположен по латеральному краю брюшной стенки. Среди животных в группе А подобных изменений не зафиксировано. При гистологическом исследовании и электронной микроскопии отмечено, что шероховатая микропористая структура проволоки из TiNi, а также биомеханическое поведение двухслойного металлотрикотажа обеспечивают оптимальную интеграцию эндопротеза в тканях организма, формируя эластичный каркас, близкий к естественному. В группе В, напротив, реакция организма, вызванная имплантированным полипропиленовым протезом, характеризовалась более выраженным фиброзом, а интеграция ткани сквозь сетчатую структуру импланта не прослеживалась.*

Заключение. *Двухслойный металлотрикотаж из TiNi, используемый для замещения сложных структур торакоабдоминальной зоны, показал многообещающие результаты, что открывает перспективы для дальнейших клинических исследований.*

Ключевые слова: *двухслойный металлотрикотаж, никелид титана, сетчатый имплант, замещение пострезекционных дефектов, интеграция в тканях, биосовместимость*

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RELEVANCE

Improvement and progress of surgical technologies have significantly expanded not only operability but also resectability in various diseases of thoracic and abdominal cavity organs. Removal of a whole organ or part of it in some cases implies a reconstructive-restorative stage, where the use of implantation technologies is vital. Considering the variants of combined surgical interventions, the most common anatomical structures that require restoration or replacement are the abdominal wall, diaphragm, pericardium, and chest wall [1]. Vast experience of such operations shows that it is not always possible to replace extensive soft tissue defects with own tissues alone. Under conditions of significant tension, the intrinsic tissues do not meet the requirements of elasticity and resilience, which leads to tension at suturing, compartment effects and the risk of primary suture failure with the development of eventration [2, 3]. The disadvantages described have necessitated the development and implementation of alternative, mesh materials with the necessary biocompatibility.

A large number of different materials and tissues have been proposed as allografts during the long history of this field development. Materials such as mersilene (Dacron), polypropylene, lavsan (Marlex), Teflon, and titanium nickelide (TiNi) have been most widely used in the fabrication of mesh implants [4–6]. The core problems of known implanted devices are due to the biomedical properties of the material [7, 8]. The material introduced into the tissues for a long period of time should have the necessary implantation characteristics, such as biocompatibility, nontoxicity, resistance to the biological environment and infection, which determines the behavior of the material in the body. In an attempt to create a universal, areactive implant, the authors modified already known materials by changing their compositions and applying various elements to the surface, but the developments have not yet found wide application [9, 10]. Most studies focus on the adaptation of the implant in the body at the cellular level, while attention is paid to physical and mechanical properties only when it comes to bone replacement. However, when plasticizing mobile structures, the material must also possess a number of properties to ensure elasticity, hydrophilicity, and strength [11]. The vast range of presented implants is due to the lack of a universal material, which forces to continue the search for improving the results of replacement and prosthetics of various structures of the thoracic and abdominal cavities.

Currently, TiNi-based implants have shown optimal results, as evidenced by their widespread use in the clinic. The advantages include bioinertness, allowing the surrounding tissues to integrate through the implant to form a single biomechanical structure with the organ. It has also been found that a passive layer of titanium oxide group is formed on the surface of TiNi alloy, which functions as a physical barrier to the oxidation of nickel, making it harmless to the human body and protecting it from corrosion [12]. And while the material itself is well researched and favored and trusted among today's practicing surgeons, progress is moving forward, and more and more new forms of weave and tissue structures made of TiNi are emerging. One such form is a two-layer knitted tape made of superelastic TiNi-wires, whose behavior in tissues and features of its integration require further study.

THE AIM OF THE STUDY

To study experimentally the morphological features of tissue integration of two-layer metal knitted TiNi mesh when replacing thoracoabdominal defects.

MATERIAL AND METHODS

Prototype implants manufactured in the laboratory of superelastic biointerfaces of National Research Tomsk State University were used as a material for the study. The implant is a two-layer tape of low-modulus superelastic TiNi-wires with a diameter of 60 μm, woven by knitting technology (Jesi knitting). The study was conducted on 40 sexually mature male Wistar rats weighing 300–350 g and aged 2.5–3.0 months. The animals were kept in the conditions of the Central Scientific Laboratory of Siberian State Medical University of the Ministry of Health of Russia. The study was conducted under the approval and supervision of the local ethics committee of Siberian State Medical University of the Ministry of Health of Russia (minutes No. 732 dd. 10/06/2020), in compliance with international and native norms of humane treatment of laboratory animals (Directive 2010/63/EU of the European Parliament and of the Council of September 22, 2010 "On the protection of animals used for scientific purposes"; Order of the Ministry of Health No. 199n dd. April 1, 2016 "On approval of rules of good laboratory practice"; Order of the Ministry of Health of the USSR No. 755 dd. August 12, 1977 "On measures to further improve the organization of work using experimental animals") [13, 14]. The experimental animals were divided into two comparison groups: in Group A ($n = 20$) the defect was replaced using a two-layer metal knitted TiNi tape, in Group B ($n = 20$) a polypropylene mesh implant was used. The choice of polypropylene mesh implant as a comparison group is due to the most frequent use of the material in the clinic for prosthetic defects localized in the thoracoabdominal areas. Preoperative preparation, surgery, anesthetic management, and postoperative management were similar in all animals. All surgical interventions were performed using zoletil-xylanite anesthesia according to the following scheme: zoletil 3 mg/kg intramuscularly (i. m.), xylanite 8 mg/kg i. m., atropine sulfate 0.1 % solution – 0.1 ml/kg subcutaneously. After anesthesia, the animals were placed and fixed on the operating table in supine position with tissue ties. The skin was treated with antiseptic solution and dried with a sterile gauze pad. The surgical site was delineated with a sterile disposable sheet.

The surgery technique. Surgical intervention was started with a midline incision on the anterior abdominal wall with a transition to the chest wall (incision length was 3–4 cm), skin flaps and subcutaneous tissue were mobilized, the musculofascial and aponeurotic flap of the anterior abdominal wall was dissected, and the processus xiphoideus and cartilaginous fragments of the arcus costalis were resected extrapleurally. As a result, a 2 × 3 cm postresection defect was formed. The endoprosthesis was cut individually according to the shape of the defect with an allowance for its edge. Fixation was performed around the perimeter using a continuous suture with 4/0 polypropylene monofilament. When the needle was passed, the animal tissue along the defect line and the edge of the endoprosthesis were captured in the suture. The implant was fixed directly to the chest wall at the level of the resected processus xiphoideus and arches of the ribs. The surgical access was sutured layer by layer, tightly with polyglycolide (PGA) 4/0 thread on an atraumatic needle. In the postoperative period, prophylaxis of wound infection by antibacterial therapy with ceftriaxone 25 mg/kg per day was performed for 7 days.

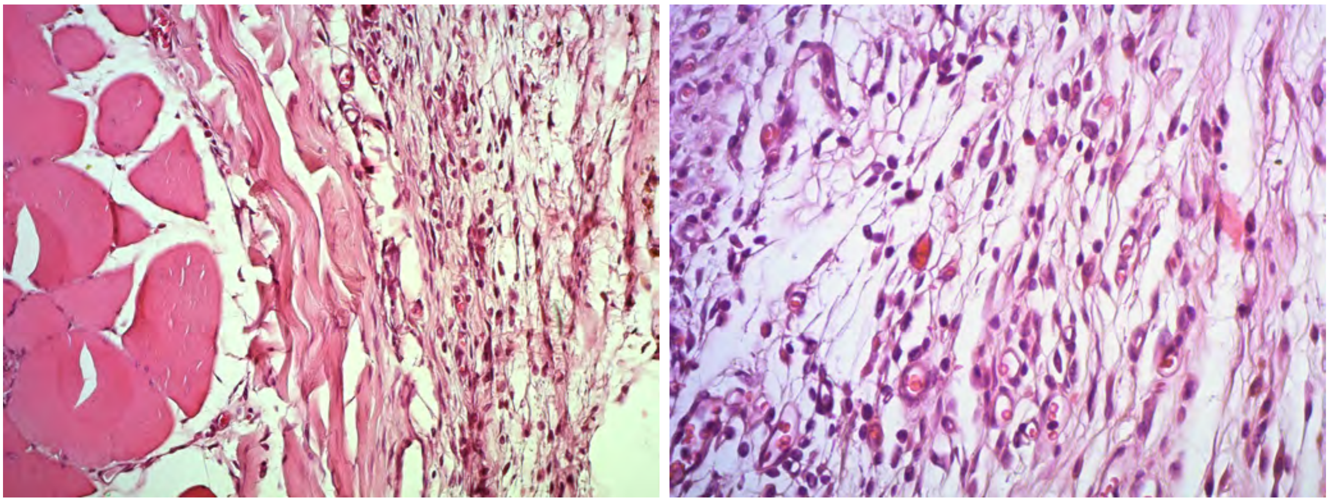
Animals were excluded from the experiment at days 14, 30, 60 and 90 after surgery by overdose of drugs used for anesthesia. After euthanasia, the abdomen and thorax of the animal were opened with a linear incision, and revision was performed. The implanted metal knitted mesh was dissected with the surrounding tissues, macroscopic structural features at the place of implant fixation to the tissues and at the areas of contact with the underlying organs were studied; the inflammatory process was evaluated. The obtained material was fixed in 10 % neutral formalin, embedded in paraffin, 5 μm thick sections were made and the preparations were stained with hematoxylin and eosin and Van-Gieson for connective tissue. Examination and microphotography were performed on an AxioLab.A1 microscope (Carl Zeiss Microscopy GmbH, Germany) with an AxioCamERc 5s video camera (Carl Zeiss Microscopy GmbH, Germany) and AxioVision Rel. 4.8 software (Carl Zeiss Microscopy GmbH, Germany). The features of tissue integration through the metal knitted mesh were evaluated by analytical scanning electron microscope. For this purpose, after preliminary fixation in 10 % neutral formalin solution, tissue fragments with implanted samples of the investigated materials were dissected. After freeze drying, the indicated samples were placed on conductive carbon tape in the electron scanning microscope chamber of Teskan Mira (Tescan Orsay Holding, Czech Republic).

RESULTS

The mean duration of surgery in Group A was 20.4 ± 3.2 min and in Group B was 21.2 ± 2.9 min. There were no statistically significant differences in terms of operation time in the groups. The animals were monitored throughout the experiment until the time of elimination. Decreased appetite and activity were clinically noted on the first day after surgery. On the day 2 after surgery, all animals had normalized motor performance and increased appetite. No intraoperative complications were noted; in both groups the surgical wound healed with primary tension, without signs of inflammation. During the growth of the animals at the standard weight gain after 30 days in four cases in Group B the occurrence of deformation at the place of implant fixation to the thoracic wall was noted; in one case the deformation area was located along the lateral edge of the abdominal wall. Among the animals of Group A no such changes were recorded.

Results of macroscopic examination. During macroscopic evaluation of the changes after the animals were eliminated from the experiment, it was noted that the deformation of the endoprosthetic zone recorded in the animals of Group B was due to hernia defects. The ligatures providing fixation of the implant to the cartilaginous parts of the ribs and in one case to the abdominal wall tissues were observed. A hernia sac containing omentum and loops of intestine was formed in the animals, in which one of the walls was represented by an implant. In addition, a significant adhesion between the walls of the sac and its contents drew attention. Among all animals in Group B, adhesions were recorded in 12 (60 %) cases and occurred after 14 days of experiment. The most frequent adhesions were located between the omentum and the implant fixation line, interintestinal adhesions were less frequent. The endoprosthetic area was characterized by stiffness, unevenness of the surface; there was a noticeable roll of scar tissue of various maturity levels along the implant fixation line, crawling along the edges on the inner surface of the mesh. In Group A, no hernia defects were detected in the endoprosthetic zone, adhesions were recorded in 3 (15 %) cases. Loose single adhesions were located between the omentum and the site of fixation of the mesh to the thoracic wall; no interintestinal adhesions were found. The endoprosthetic area was characterized by elasticity and was easy to deform. Starting from the day *14* of the experiment, the occurrence of a thin tissue film on the inner surface of the mesh was noted, evenly filling the pores of the mesh material, which hardly allowed to separate the implant from the tissue graft.

Results of histological examination. At the histological examination on the day *14* at the operation site in both groups there were still signs of acute inflammatory reaction manifested by infiltration with neutrophilic leukocytes, lymphocytes and macrophages; at that, in Group B the infiltration phenomena were more pronounced, the cells were concentrated not only around the mesh elements, but also diffusely. In Group A, the inflammatory infiltration was predominantly lympho- and macrophagal, and single neutrophils were found only near the mesh elements. A granulation tissue consisting of thin collagen fibers, small blood vessels and cellular elements, mainly fibroblasts, lymphocytes and macrophages, was formed between the mesh and the adjacent layers of the anterior abdominal wall muscles (Fig. 1). In both groups, edema was observed in the endomysium of the skeletal muscles in contact with the implants located in separate visual fields.



a ***b***

FIG. 1.

__a__ – granulation tissue around muscle fibers 14 days after implantation of the titanium nickelide metal knitted mesh. __b__ – vessels and cellular elements (lymphocytes, macrophages, fibroblasts) of granulation tissue after implantation of titanium nickelide metal knitted mesh. Haematoxylin and eosin staining. Magnification ×400 (__a__), ×600 (__b__)

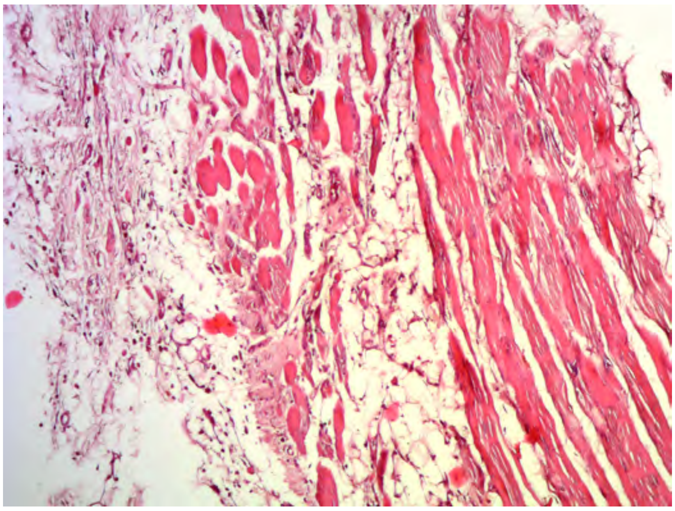


FIG. 2.

Tissue graft 30 days after implantation of the polypropylene mesh. Haematoxylin and eosin staining. Magnification ×400

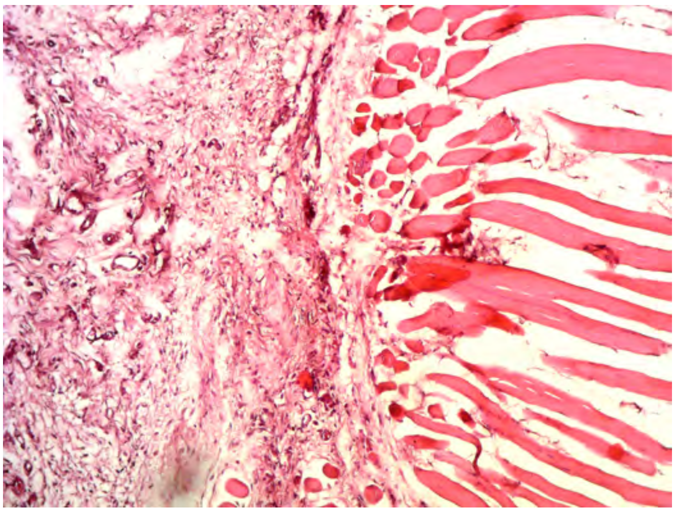


FIG. 3.

The tissue graft 30 days after implantation of titanium nickelide metal knitted mesh. Haematoxylin and eosin staining. Magnification ×400

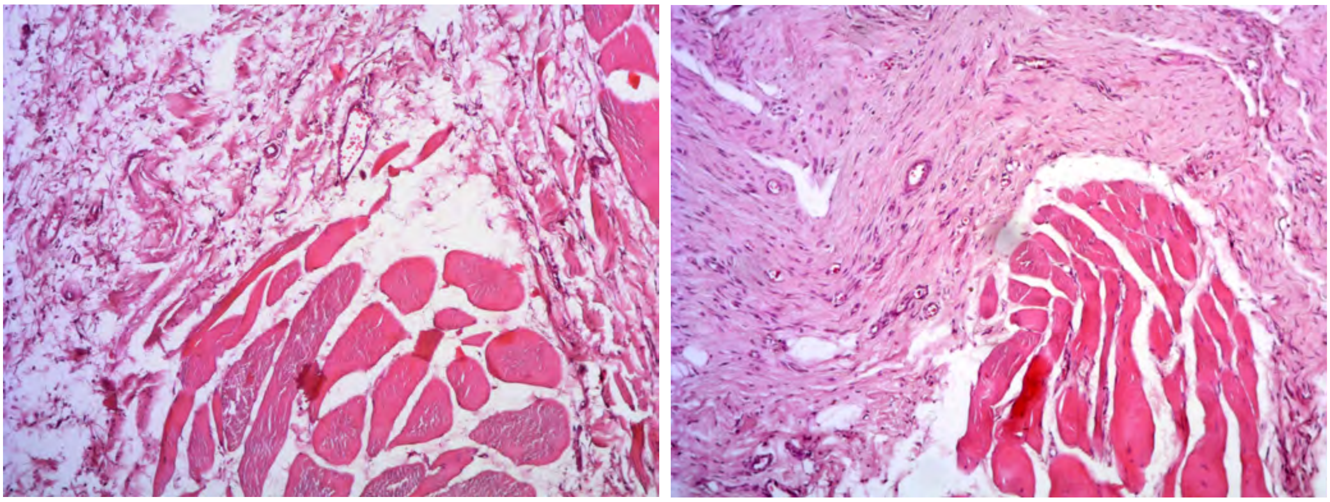
By the day 30 in both groups, the number of leukocytes in the infiltrate decreased and the number of fibroblasts increased, while the granulation tissue on the implant surface differed only in the degree of maturity. In Group B, areas of neutrophil aggregation persisted and collagen fibers were located along the mesh elements, the orientation was indistinct (Fig. 2).

In Group A, the number and thickness of collagen fibers increased, as a result of which they acquired a characteristic orientation along the TiNi strands and formed bundles, repeating the structure of the implant (Fig. 3). Growth of fibrocartilaginous tissue was observed along the edge of the resected cartilages.

On the day 60 in Group B there were still signs of edema and body response to foreign tissue, manifested by areas of lymphocyte and macrophage accumulation (Fig. 4a). In Group A, a graft of mature connective tissue formed around the implant (Fig. 4b).

In Group A, fibroblast growth was observed through the mesh knitted structure of the nickelide-titanium implant with tissue graft filling the thread pores (Fig. 5).

By the end of the study, a capsule of mature connective tissue was formed around the implants in both groups, which in Group B was characterized by the presence of areas of granulation tissue at the place of contact with the implant. In Group A the connective tissue capsule had a less pronounced thickness with the phenomena of filling with fibroblasts and collagen fibers of the implant mesh structure. The organ-specific differentiation of the tissues of the newly formed graft was observed along the implant fixation line.



a ***b***

FIG. 4.

a *– loose connective tissue between muscle fibers of the abdominal wall 60 days after the implantation of polypropylene mesh.* ***b*** *– mature connective tissue around the muscle fibers of the graft 60 days after the implantation of titanium nickelide metal knitted mesh. Haematoxylin and eosin staining. Magnification ×400*

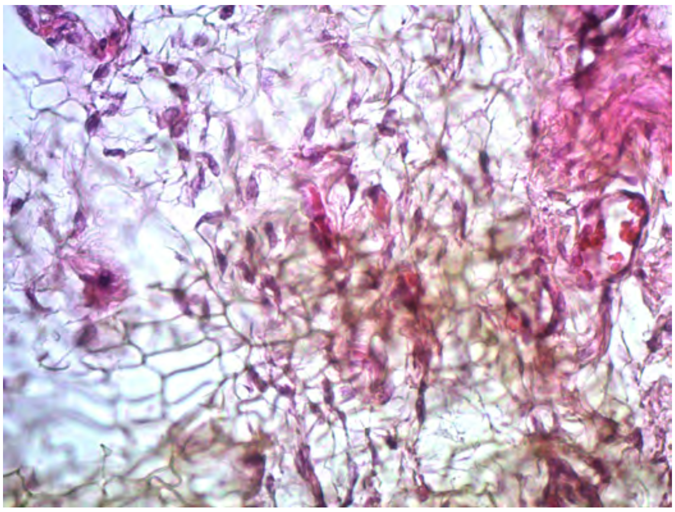


FIG. 5.

Fibroblasts filling the mesh structure of the nickelide-titanium implant on day 60 of the experiment. Haematoxylin and eosin staining. Magnification ×600

Mature connective tissue is visible between the muscle fibers. Muscle buds were observed at the site of muscle fiber incision as a manifestation of elements of skeletal muscle fiber regeneration.

Electron microscopy results. The study of preparations using electron microscopy in Group B indicated the absence of strong bonds between the connective tissue graft and the polypropylene mesh. The cells, as well as intercellular elements, were accommodated in the form of a dense roll around the perimeter of the implant with areas of crawling on the implant along the smooth filaments, ending with the intertwining of fibers to form a roll (Fig. 6). The integration of tissue elements through the mesh structure of the implant was not observed.

Group A showed a different microscopic pattern from day 14 onwards. The formation of connective tissue regenerate began at the areas of intersection and contact of TiNi-filaments as clusters of fibroblasts and bundles of collagen fibers forming plexuses of various types (Fig. 7). The surface of the tissue graft acquired a reticulated shape, repeating the contour of the implant. By the day 30 collagen fibers and fibroblasts in some areas completely filled the pores of the two-layer implant.

By the day 90 after surgery in Group A, the strength of the implant connection with the thoracic wall tissues increased significantly. At the level of the remaining ends of the resected cartilages, a roll was formed consisting of the formed fibrocartilaginous tissue, the embryo

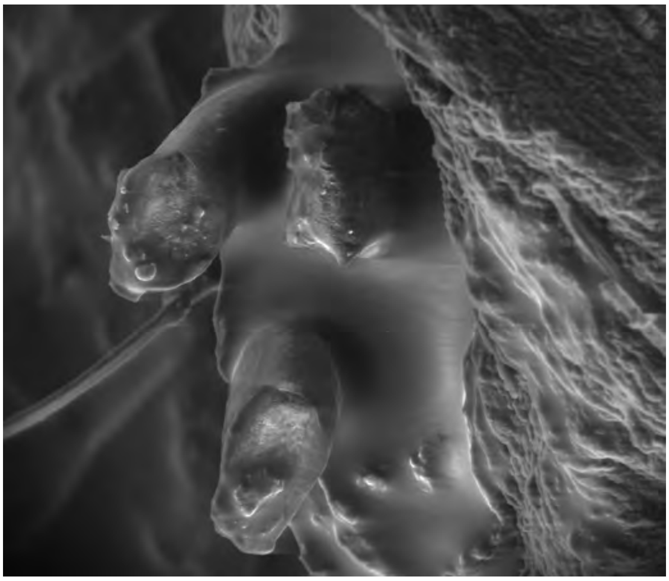


FIG. 6.
The formation of a cell roll along the polypropylene filaments in Group B on day 14. Scanning electron microscope. Magnification ×350

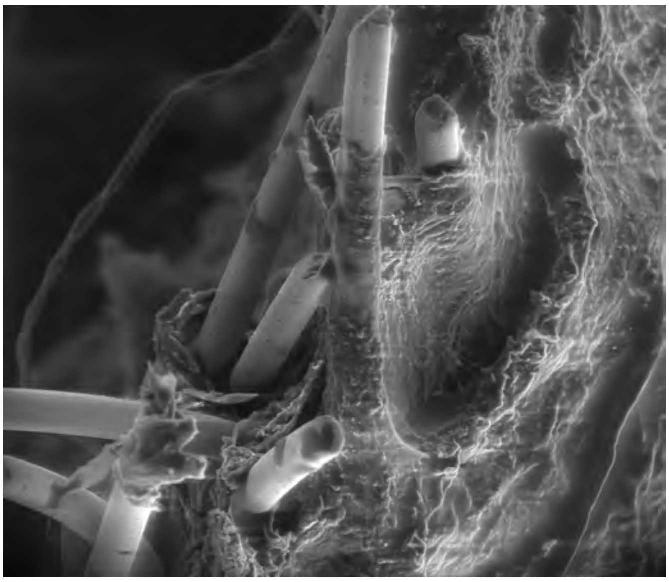


FIG. 7.
Formation of connective tissue graft in Group A on day 14. Scanning electron microscope. Magnification ×350

of which is the remaining supracartilage of the arcus costalis. It is observed that the cartilaginous tissue intimately adjoins and as if "crawls" as a part of the connective tissue graft on the implant surface, and the special form of fusion in this area due to the intertwining and sprouting of the directed connective tissue bundles through the mesh structure provides the stability and strength of the connection. In Group B no such effect was observed: fibrous cartilaginous tissue formed a regenerative roll and fixed the implant by means of a connective tissue bridge.

DISCUSSION

Choosing an endoprosthesis to replace complex body areas, especially those such as thoracoabdominal junctions, is challenging. For the replacement of small prolapses it is possible to use any type of endoprosthesis or enough own tissues, it is much more difficult to find an effective material for the elimination of extensive defects in which the edges are represented by different anatomical structures and tissues [15–17]. Advanced technologies in the development of medical-grade materials have increased the level of demands being imposed on the biomechanical properties of the material, namely the ability to withstand the varying load applied to the implant. Such a capability determines its functionality and affects immediate and long-term results. The difference in the stress-strain state between the implant and the body tissues causes complications manifested as hernia defects. In our study, no such complications were observed in Group A, which is determined by the extensibility of the tissue – implant complex. Such an effect, with its high tensile strength, plays an important role when the body area increases, as for example, when the patient gains excess weight or grows the body. However, despite all the physical and mechanical advantages, biocompatibility is the decisive factor when selecting a material as a bioprosthesis for defect replacement in humans.

The main task of any mesh implant is to become the basis for connective tissue graft, and in the absence of biocompatibility properties, its physical and mechanical qualities will not allow it to unfold. If the quantity and quality of collagen fibers depend on the biocompatibility properties during the formation of tissue graft, such parameters as the structure and thickness of the thread as well as the size of the weave mesh play a key role in the fixation of the implant and determine the risk of hernia defects. The results of our study convincingly prove that smooth surface architecture is not a favorable condition for the formation of a tissue graft united with the implant, preventing cell adhesion and reducing the overall biocompatibility. Such conditions cause the body to react to the implant as a foreign body, resulting in the formation of keloids, chronic pain syndrome and hernia defects. Similar behavior was demonstrated in the polypropylene mesh group in the form of long-lasting inflammation around the implant, stimulating the growth of excess connective tissue, isolating the implant in the body. At the same time, in Group A, the porous structure of the filament and the two-layer nature of the TiNi implant favored cell proliferation. This manifests itself as a cellular response to surface topography and is a primary feature of the formation of many tissues. In addition, the rough structure allows fibroblasts from the implant surface to be integrated into the bilayer structure, forming a single tissue graft that has elastic properties with an optimal safety margin.

CONCLUSION

Experimental administration of an endoprosthesis made of two-layer TiNi knitted mesh has shown promising results. In the group that used the polypropylene implant, more hernia defects were observed and adhesions were more frequently observed. In addition, the body response induced by the implanted polypropylene prosthesis was characterized by more pronounced fibrosis, and tissue integration through the mesh structure of the implant was not observed. The porous structure of the TiNi-wire and the biomechanical and biochemical properties of the two-layer knitted mesh provide optimal integration of the endoprosthesis in the body tissues and contribute to the formation of an elastic framework close to the natural one. two-layer TiNi knitted mesh in the replacement of complex structures of the thoracoabdominal area has shown promising preliminary results, which opens prospects for further clinical studies, including the use of methods of evaluative morphometry.

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Conflict of interest

The authors declare the absence of a conflict of interest.

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