Morphological Characteristics of Tissue Reaction in the Zone of "Cousin" Unwoven Polypropylene Endoprosthesis Implantation

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Comparative histological and electron microscopic study of mouse tissues at the site of implantation of Cousin mesh and unwoven polypropylene endoprostheses was carried out. Implantation of unwoven endoprosthesis was associated with more pronounced inflammatory reaction with longer elimination of neutrophilic granulocytes and macrophages from the adjacent tissues. The growth of connective tissue components between the implant fibers with the formation of fine connective tissue capsule enveloping the prosthesis was observed during earlier periods of the experiment.

Key Words: *implantation; cell reaction; unwoven polypropylene endoprostheses*

External hernias of the anterior abdominal wall belong to the most prevalent surgical diseases detected in 3-7% population [8]. More than 20 million herniotomies are annually performed in the world, this amounting to 10-15% of all surgical interventions [5,13]. Non-stretching methods of abdominoplasty with synthetic endoprostheses are more and more often used in modern herniology [2]. This led to improvement of the treatment efficiency, especially in patients with pronounced concomitant diseases [4].

Mesh polypropylene endoprostheses are most often used in herniology [6]. Polypropylene causes the development of intensive inflammatory reaction, which leads to rapid and tight growth of the implant into the adjacent tissues [1]. On the other hand, it leads to the formation of a fibrous capsule and cicatrices around the implant, which promotes its contraction and deformation [11,14]. It often becomes the cause of adhesive process in the abdominal cavity [7]. It was found that the intensity of inflammatory reaction around the endoprosthesis directly depends on its physical properties: compactness, size of pores, knitting pattern [6,9].

We carried out a comparative morphological study of the tissue reaction to implantation of "Cousin" mesh and unwoven polypropylene endoprostheses (Cousin Biotech).

MATERIALS AND METHODS

Experiments were carried out on 60 albino male of the same age, which were implanted polypropylene endoprostheses under the skin on the back. The animals were divided into control and two experimental groups, 20 per group (5 mice per stage of observation). Cousin polypropylene mesh implants (Biomesh P1 Mesh; experimental group 1) and Cousin unwoven polypropylene endoprostheses (Biomesh NK 2 Mesh; experimental group 2) were implanted under the skin of the back onto the muscles to narcotized animals (0.5 ml sodium thiopental, 1:10) under sterile conditions. The implants were

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not fixed; interrupted suture was applied on the skin with Biosin 4/0 thread. No implantation was carried out in the control group. The animals were sacrificed 3, 7, 14, and 28 days after surgery and the material for morphological study was collected (tissue from the site of implantation together with the endoprosthesis). All manipulations were carried out in accordance with "Regulations for Studies with the Use of Experimental Animals".

Tissue fragments with the implant were fixed in 10% neutral formalin. Histological studies were carried out on paraffin sections (5 μ) stained with hematoxylin and eosin. Quantitative evaluation of cell composition was carried out using a MEKOS-C television image analyzer, the data were processed using Statistica software. Samples for electron microscopy were fixed in 2.5% glutaraldehyde and 1% osmium tetroxide and dehydrated in ascending alcohols, after which were embedded in epon-araldite mixture. Ultrathin sections were examined under a Phillips CM-10 electron microscope.

RESULTS

Histological study of soft tissues in control mice showed moderate edema and minor lymphocyte infiltration.

Complex morphological (histological and ultrastructural) study of tissues from the endoprosthesis implantation zone showed that 3 days after surgery the cell reaction to the mesh endoprosthesis was mainly presented by monocytes-macrophages in the presence of moderate amounts of neutrophilic granulocytes and lymphocytes and negligible counts of acidophilic (eosinophilic) granulocytes and mast cells (Fig. 1, *a*). The neutrophilic-macrophagic reaction of tissues was observed in experiments with unwoven endoprosthesis; lymphocyte count was also high, eosinophilic and mast cells were solitary. Fibroblast counts were minimum in both groups. It is noteworthy that neutrophil count after implantation of unwoven endoprosthesis was 2.7 times ($p<0.05$) higher than after mesh implantation. Electron microscopy showed

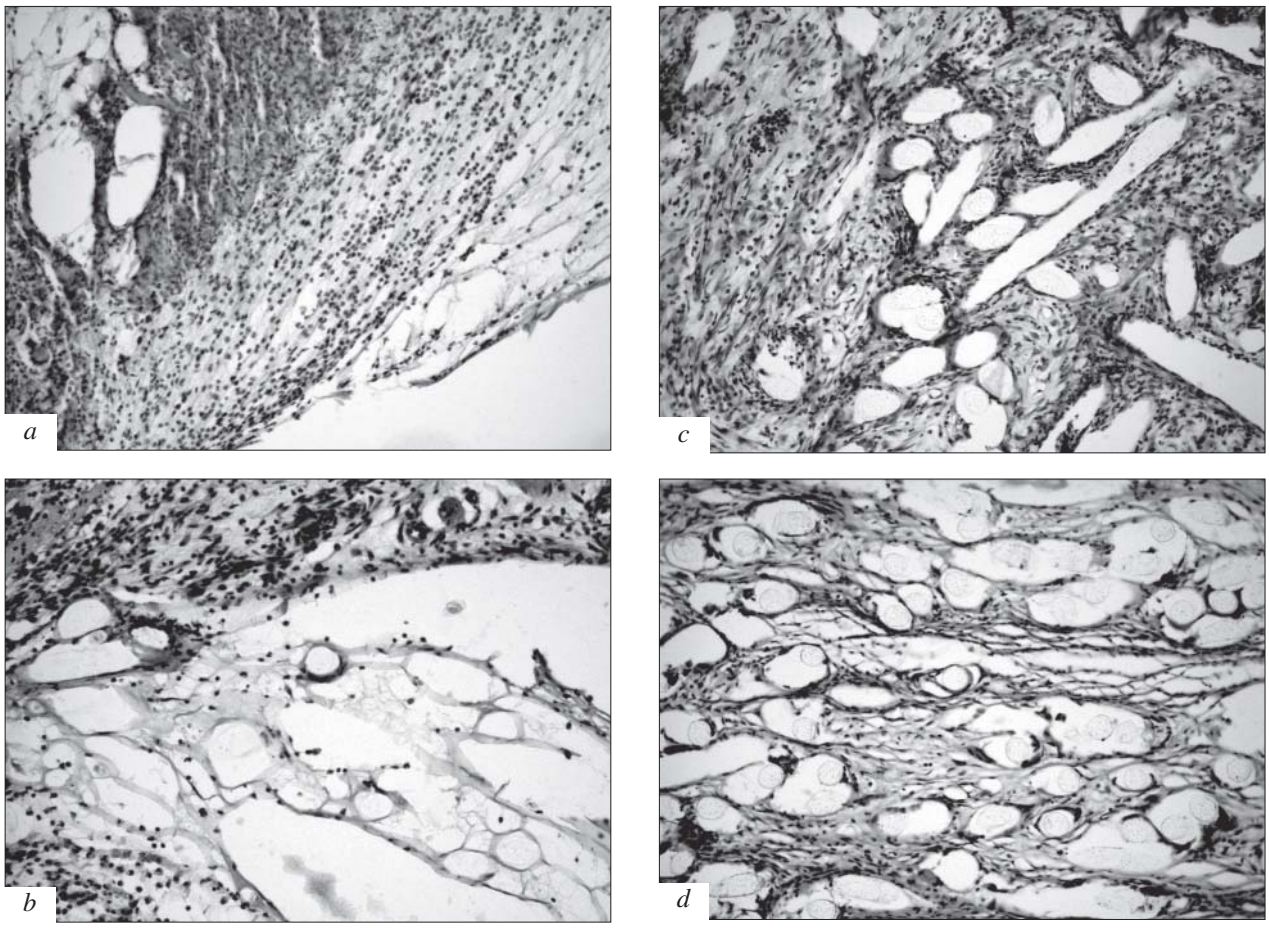

Fig. 1. Histological changes in tissues 3 (*a*), 7 (*b*), 14 (*c*), and 28 days (*d*) after implantation of mesh (*a*) and unwoven (*b*, *c*, *d*) Cousin polypropylene endoprostheses. Hematoxylin and eosin staining, ×200.

numerous neutrophilic granulocytes containing specific granules (Fig. 2, *a*).

By day 7 of the experiment, the count of fibroblasts in animals implanted mesh endoprosthesis sharply increased (37.5 times in comparison with the previous term, $p<0.01$) and fine collagen fibers were seen in the zone of contact with the prosthesis. Fibroblast count virtually did not change at the site of implantation of the unwoven endoprosthesis, but the cells were functionally active judging from dilated cisterns of the endoplasmic reticulum (Fig. 2, *b*). The fibers of the implant were surrounded by solitary collagen fibrils and fibrin-like substance containing inflammation cells (neutrophilic granulocytes, macrophages, lymphocytes, mast cells). Neutrophil count decreased by 31% ($p<0.05$) in comparison with the previous term in the unwoven endoprosthesis group and by 14% ($p<0.05$) in the mesh group. The counts of macrophages and lymphocytes somewhat decreased in the mesh endoprosthesis group and slightly increased in the unwoven material group (Fig. 1, *b*). Morphological signs of macrophage activation were noted: the presence of multiple cytoplasmic processes branching and anastomosing with each other and numerous phagosomes, granules, and vesicles. The granular endoplasmic reticulum in these cells was presented by short and narrow profiles. Numerous

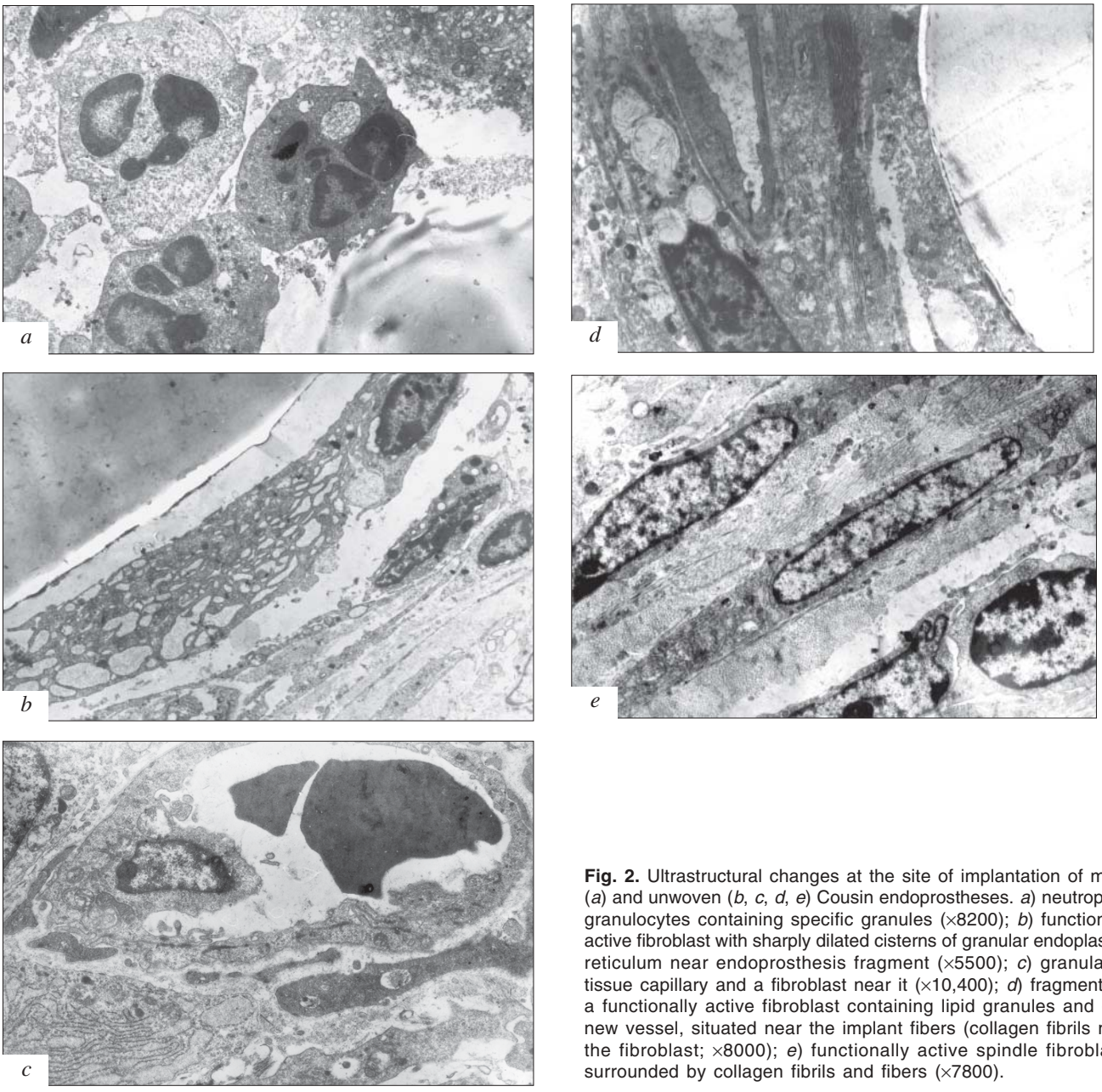

Fig. 2. Ultrastructural changes at the site of implantation of mesh (*a*) and unwoven (*b*, *c*, *d*, *e*) Cousin endoprostheses. *a*) neutrophilic granulocytes containing specific granules (×8200); *b*) functionally active fibroblast with sharply dilated cisterns of granular endoplasmic reticulum near endoprosthesis fragment (×5500); *c*) granulation tissue capillary and a fibroblast near it (×10,400); *d*) fragments of a functionally active fibroblast containing lipid granules and of a new vessel, situated near the implant fibers (collagen fibrils near the fibroblast; ×8000); *e*) functionally active spindle fibroblasts surrounded by collagen fibrils and fibers (×7800).

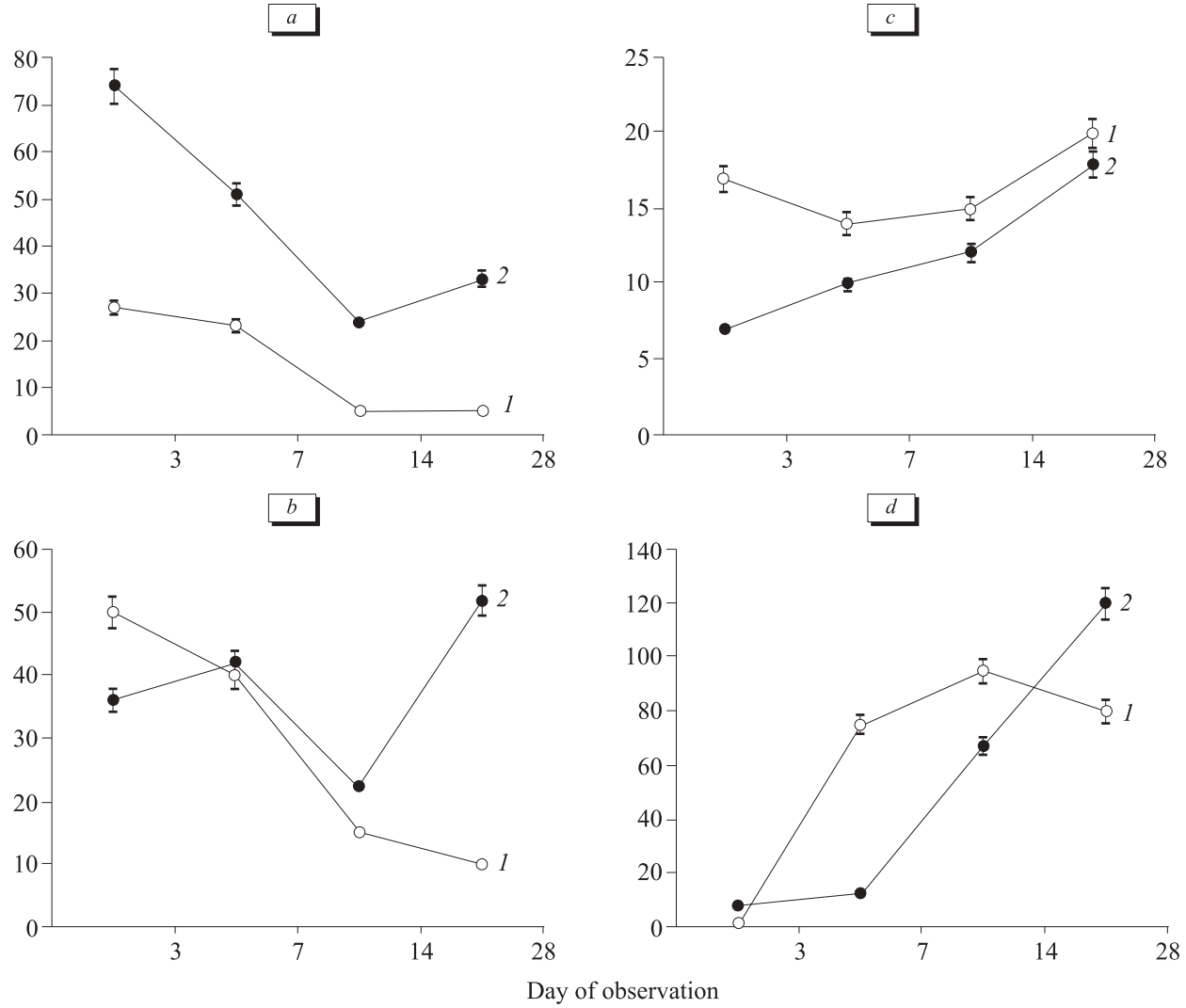

Fig. 3. Dynamics of cell composition at the site of implantation of mesh (*1*) and unwoven (*2*) Cousin polypropylene endoprostheses. *a*) neutrophilic granulocytes; *b*) macrophages; *c*) lymphocytes; *d*) fibroblasts. Ordinate: cell number in 10 fields.

mitochondria with clarified matrix and impaired orientation of cristae were seen. The nucleus of irregular shape was displaced towards the periphery. Histological study of preparations from animals of both groups showed many new capillaries, their number was higher in the mesh prosthesis group. Electron microscopy showed vascular endotheliocytes with large nuclei, chromatin condensed along the nucleolemma, plasmalemma forming pseudopodias and cytoplasmic processes directed into the vascular lumen; round mitochondria with clear matrix and reduced number of cristae were noted. Large and small invaginations of the plasmalemma led to the appearance of vesicles on the side facing the lumen and facing the tissue, this indicating intensification of the transport processes (Fig. 2, *c*).

After 14 days, continuing reduction of macrophage and neutrophil counts was paralleled by an increase in the fibroblast count (Fig. 3, *d*); the count of these cells increased 5.6 times ($p<0.01$) in comparison with day 7 in the unwoven prosthesis group and 1.3 times ($p<0.05$) in the mesh implantation group. However, the total count of fibroblasts in the zone of implantation of the unwoven prosthesis at this term of the experiment was 30% lower ($p<0.05$) in comparison with the mesh endoprosthesis. Implantation of the mesh endoprosthesis was also associated with an increase in the number and thickness of collagen fibers, some these fibers starting to grow between the implant fibers in some sites. The fibroplastic reaction was different after implantation of unwoven polypropylene endoprosthesis: a fine connective tissue capsule was seen at the implant periphery against the background of pronounced tissue edema. In addition, there were many loose collagen fibers and fibroblasts between the

endoprosthesis elements, virtually each polypropylene fiber was surrounded by collagen fibrils forming a capsule (Fig. 1, *c*). Ultrastructural study showed poorly differentiated and proliferating fibroblasts; their granular endoplasmic reticulum was presented by parallel tubules, the Golgi complex was located near the nucleus and was surrounded by small and large vesicles. Numerous mitochondria with clarified matrix were seen. The nucleus was located eccentrically; chromatin was evenly distributed in the entire volume of the nucleus. Collagen fibers forming bundles were located mainly in the zone of contact with the implant (Fig. 2, *d*).

By the end of the experiment (28 days) fibroblasts predominated in histological preparations in the zone of implantation of both endoprostheses. Devastation of vessels was seen. A wide connective tissue capsule enveloped the mesh implant, the greater part of the implant cells being also replaced by the connective tissue. The connective tissue capsule enveloping the unwoven prosthesis was much thinner and the connective tissue components totally grew into the implant (Fig. 1, *d*). Electron microscopy showed horizontally oriented fibroblasts surrounded by collagen fibers (Fig. 2, *e*). Moderate diffuse infiltration in the zone of endoprostheses implantation persisted in both groups. These differences in tissue reaction seem to be caused by differences in the types of implanted endoprostheses. Cousin mesh endoprosthesis (Biomesh P1 Mesh) is a knit polypropylene implant with surface density of 100 g/m^2 and 0.6 mm fiber. The fibers of unwoven Cousin endoprosthesis (Biomesh NK 2 Mesh) are connected in a special way under conditions of thermal processing and form no weaves and nodes, in contrast to the mesh implant, whose fibers form nodular connections during the mesh knitting. This structure of unwoven prosthesis provides better growth of connective tissue components between the implant fibers and renders it resistance to stretching and deformation. Moreover, the amount of polypropylene during making an unwoven endoprosthesis is reduced to 35 g/m^2, while the thickness of its fibers is just 0.25 mm, which is also essential for tissue reaction.

According to published data [10], long presence of the majority of mesh endoprostheses in tissues causes the development of acute/subacute inflammation with moderate edema and infiltration by polymorphonuclear leukocytes and macrophages during 1-3 weeks. These changes are most pronounced after 14-21 days, after which they transform into a more or less manifest chronic inflammation, surrounding the foreign body. This is paralleled by the formation of cicatricial tissue containing numerous collagen fibers and forming a 3D-structure around and inside the mesh implant. The fibroblast count and vascularization level are in inverse correlation with the duration of inflammatory reaction, while manifest acute inflammation at the initial stages of implantation causes intensive development of fibrous tissue [3].

The most significant factors essential for biocompatibility of foreign materials are the intensity and duration of inflammatory reaction and healing processes, which, in turn, depend on the type of material [12]. The severity of inflammatory reaction to introduction of biological material is eventually essential for the growth through the endoprosthesis in the implantation zone. In addition, the formation of connective tissue in this zone directly depends on the intensity of inflammatory reaction. The duration of the foreign body type reaction to implantation depends on the volume of implanted material. It was found that biomaterials with larger pores and lesser volume of the polypropylene component cause less pronounced inflammatory reaction and fibrosis, are characterized by microscopic signs of normal healing and better integration in the adjacent tissues in comparison with the reaction to a mesh implant with high content of polypropylene [9].

Hence, we detected differences in the dynamics of tissue reaction to implantation of mesh and unwoven polypropylene endoprostheses. Implantation of the unwoven endoprosthesis caused a more pronounced inflammatory reaction of tissues. The growth of connective tissue components between the implant fibers with the formation of a fine connective tissue capsule enveloping the prosthesis was observed during earlier period of experiment. Therefore, both endoprostheses are recommended for further clinical studies for their use in non-stretching plastic repair of the abdominal wall in hernias.

REFERENCES

1. E. A. Dubova, I. A. Chekmaryova, A. I. Shchyogolev, *et al.*, *Byull. Eksp. Biol. Med.*, **129**, No. 5, 590-595 (2006).
2. V. N. Yegiev, M. N. Rudakova, and M. V. Svatkovskii, *Khirurgiya*, No. 6, 18-22 (2000).
3. D. S. Sarkisov, E. G. Kolokol'chikova, R. I. Kaem, and A. A. Pal'tsyn, *Arkh. Patol.*, No. 1, 9-15 (1989).
4. A. D. Timoshin, A. V. Yurasov, and A. L. Shestakov, *Gerniologiya*, No. 1, 5-10 (2004).
5. V. D. Fyodorov, A. A. Adamyan, and B. Sh. Gogiya, *Khirurgiya*, No. 1, 11-14 (2000).
6. W. S. Cobb, K. W. Kercher, and B. T. Heniford, *Surg. Innov.*, **12**, No. 1, 63-69 (2005).
7. R. C. Dinsmore, W. C. Calton Jr., S. B. Harvey, and M. W. Blaney, *J. Am. Col. Surg.*, **191**, No. 2, 131-136 (2000).
8. B. T. Heniford, A. Park, B. J. Ramshaw, and G. Voeller, *Ann. Surg.*, **238**, No. 3, 391-399 (2003).

9. U. Klinge, B. Klosterhalfen, V. Birkenhauer, *et al.*, *J. Surg. Res.*, **103**, No. 2, 208-214 (2002).
10. B. Klosterhalfen, K. Junge, B. Hermanns, and U. Klinge, *Brit. J. Surg.*, **89**, No. 8, 1043-1048 (2002).
11. G. E. Leber, J. L. Garb, A. I. Alexander, and W. P. Reed, *Arch. Surg.*, **133**, No. 4, 378-382 (1998).
12. R. Rosch, K. Junge, A. Schachtrupp, *et al.*, *Eur. Surg. Res.*, **35**, No. 3, 161-166 (2003).
13. V. Schumpelick, U. Klinge, G. Welty, and B. Klosterhalfen, *Chirurg.*, **70**, No. 8, 876-887 (1999).
14. M. Van't Riet, J. W. Burger, F. Bonthuis, *et al.*, *Surg. Endosc.*, **18**, No. 4, 681-685 (2004).