Morphological Changes in the Implantation Zone of Prolen and Esfil Reticular Endoprostheses

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Comparative histological and electron microscopic study of tissues in the zone of implantation of polypropylene endoprostheses Prolen and Esfil was carried out in mice. Implantation of a reticular endoprosthesis Esfil was associated with a more pronounced infiltration with neutrophilic granulocytes, macrophages, and lymphocytes. Both nets were characterized by pronounced integration in the adjacent tissues in the implantation zone and can be recommended for clinical use.

Key Words: *implantation; cellular reaction; reticular endoprostheses*

The problem of surgical treatment of external hernias of different location remains unsolved. Today hernias are regarded not as a local pathological process in the anterior abdominal wall, but as a complex polyetiological disease leading to multiple visceral dysfunctions [2]. Problems associated with hernias are significant from medical, social, and economic viewpoints. According to statistical data, 2-4% population suffers from abdominal wall hernias and 4-11% patients develop postoperative hernias after laparotomy [7]. Hernioplasty is annually carried out in about 180,000 patients in Russia, in 280,000 in Germany, and in more than 500,000 patients in the USA [5,6,8]. Hernioplasty involves obligatory fortification of the anterior abdominal wall, and therefore synthetic implants are more and more often used in the treatment of hernias [1,2, 10,12]. Today polypropylene is the most prevalent synthetic transplant in surgery of hernias.

We studied the morphology of tissue reaction to implantation of PROLEN (Ethicon) and ESFIL (Linteks) nets.

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MATERIALS AND METHODS

Experimental study was carried out on 60 male mice of the same age, implanted reticular prostheses subcutaneously on the back. The animals were divided into control and two experimental groups, 20 mice per group (5 mice per point). Reticular polypropylene implants Prolen (experimental group 1) and Esfil (experimental group 2) were implanted to narcotized animals (0.5 ml sodium thiopental, 1:10, peritoneally) under the skin on the dorsal muscles under sterile conditions. The implants were not fixed: separate nodular sutures were made on the skin with a Biosin 4/0 thread. Controls received no implantation. On days 3, 7, 14, and 28 after the intervention the animals were sacrificed and material (tissue from the implantation area with the endoprosthesis) was resected for morphological study.

Tissue fragments with the implant were fixed in 10% neutral formalin. Histological study was carried out on 5-μ paraffin sections stained by hematoxylin and eosin. Quantitative evaluation of cellular composition was carried out on a MEKOS-91 television image analyzer. Specimens for electron microscopy were fixed in 2.5% glutaraldehyde and 1% osmium tetroxide. Dehydration was carried out in ascending alcohols, after which the sample was em-

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bedded in epon-araldite. Ultrathin sections were examined under a Philips CM-10 electron microscope.

The data were statistically processed using Statistica software.

RESULTS

Histological study of soft tissue from control mice showed moderate edema and lymphocyte infiltration.

Complex morphological (histological and ultrastructural) study of tissues from the zone of implantation of reticular endoprostheses (Prolen and Esfil) showed differences in the time course and pattern of cellular reaction. On day 3 after implantation of Prolen net we observed primarly macrophagic and lymphocytic reaction, while implantation of Esfil induced neutrophilic and macrophagic reaction. Solitary fibroblasts, fine loose collagen fibers, and a moderate number of new vessels were detected around both nets (Fig. 1, *a*, *b*). Ultrastructural study showed functionally active macrophages with numerous cytoplasmic axons, multiple lysosomes, lipid and micropinocytic vacuoles. Their phagosomes contained tissue detritus (Fig. 2, *a*).

By day 7 tissue reaction was somewhat different: formation of granulation tissue was observed. The content of neutrophilic granulocytes dropped 6.1 times ($p<0.01$) after implantation of Prolen and 5.9 times ($p<0.01$) after Esfil implantation. The number of fibroblasts and collagen fibers increased significantly in animals implanted Prolen (fibroblast number increased 2.7 times, $p<0.01$) and in those with Esfil net (3.5 times, $p<0.01$). However, the absolute count of cells was higher in animals implanted Prolen. Collagen-producing fibroblasts had electron-dense cytoplasm, granular endoplasmic reticulum occupied virtually its entire volume (Fig. 2, *b*). Fine collagen fibrils near fibroblasts (a result of their specific collagen-producing function) attest to activity of these cells [11]. Numerous new vessels were seen around the nets; endothelial cells of these vessels had large nuclei with even contour.

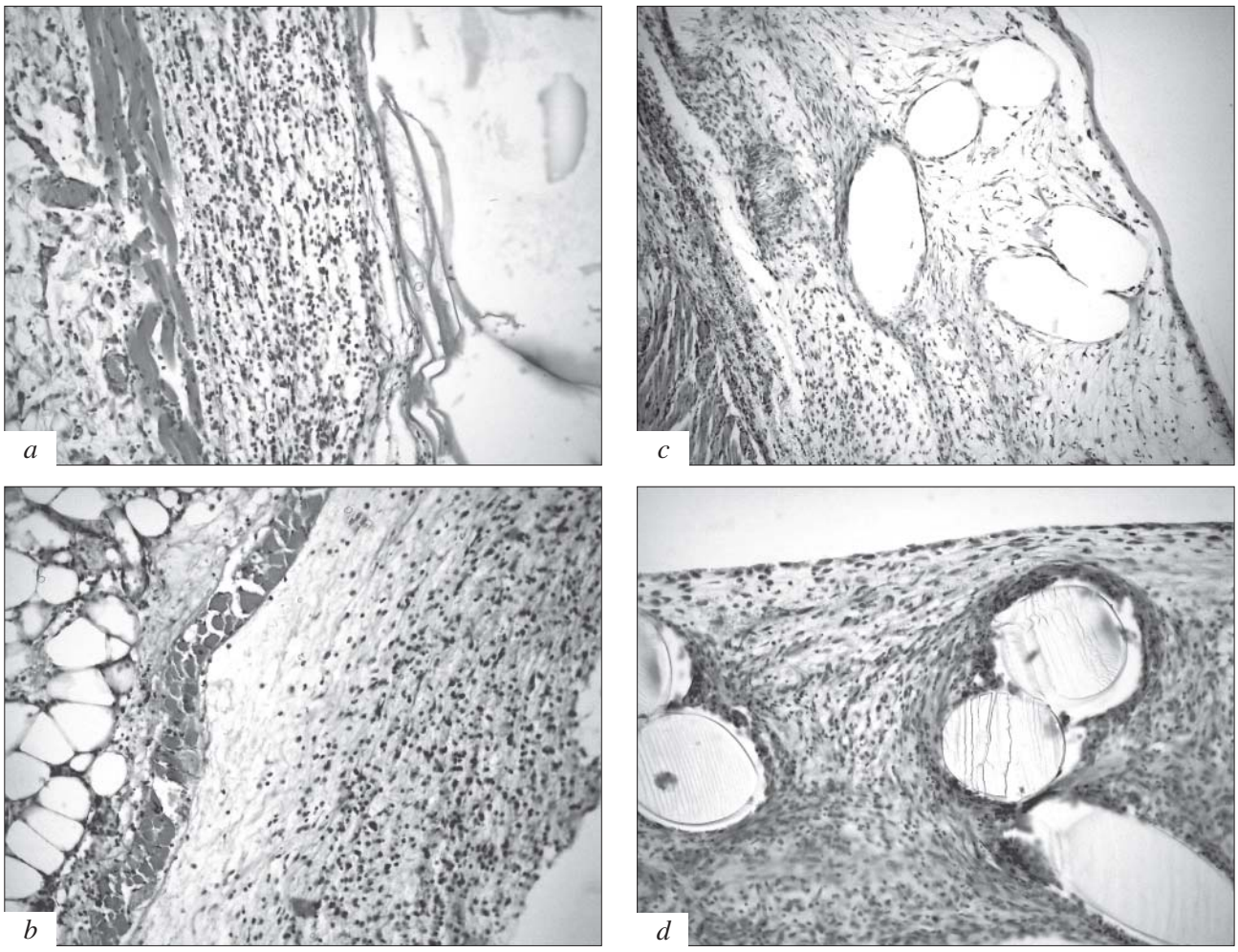

Fig. 1. Histological changes in tissues 3 (*a*, *b*) and 14 (*c*, *d*) days after implantation of Prolen (*a*, *c*) and Esfil (*b*, *d*). Hematoxylin and eosin staining, ×200 (*a*, *b*, *d*); ×100 (*c*).

Chromatin was condensed along the nucleolemma, plasmalemma formed pseudopodias and cytoplasmic processes directed into the vascular lumen; mitochondria were round or oval, more often with clarified matrix and lesser number of cysts. Granular endoplasmic reticulum was presented by short membrane profiles. Large and small invaginations of the plasmalemma led to emergence of vesicles on the side facing the lumen and on the side facing the tissue, this indicating intensification of transport processes (Fig. 2, *c*). Connective tissue components start growing into the implant cells during this period.

After 14 days numerous collagen fibers were seen in the presence of a moderate count of fibroblasts (1.5 times less *vs.* the previous stage of experiment, $p<0.01$, in cases with Prolene implantation and 87% more, $p<0.05$, in cases with Esfil implantation). Collagen fibers were thicker than during the previous stage, were positioned parallel to the implant and grew between its fibers, forming a delicate capsule with new vessels around the endoprostheses (Fig. 1, *c*, *d*). Pronounced lymphocytic macrophagic infiltration of tissues with solitary neutrophils was still seen during this period around endoprostheses in animals implanted Esfil net (Fig.

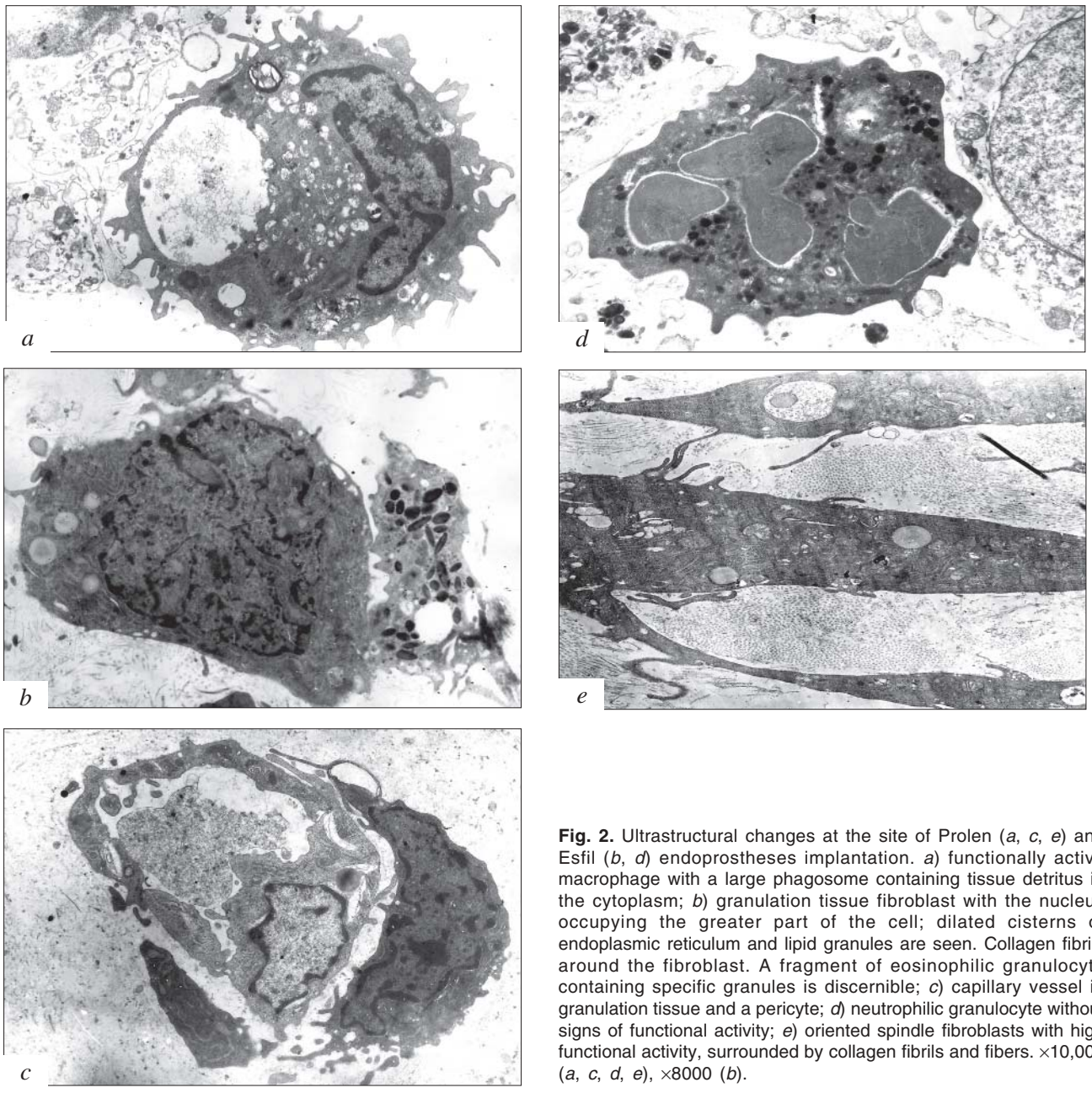

Fig. 2. Ultrastructural changes at the site of Prolen (*a*, *c*, *e*) and Esfil (*b*, *d*) endoprostheses implantation. *a*) functionally active macrophage with a large phagosome containing tissue detritus in the cytoplasm; *b*) granulation tissue fibroblast with the nucleus occupying the greater part of the cell; dilated cisterns of endoplasmic reticulum and lipid granules are seen. Collagen fibrils around the fibroblast. A fragment of eosinophilic granulocyte containing specific granules is discernible; *c*) capillary vessel in granulation tissue and a pericyte; *d*) neutrophilic granulocyte without signs of functional activity; *e*) oriented spindle fibroblasts with high functional activity, surrounded by collagen fibrils and fibers. ×10,000 (*a*, *c*, *d*, *e*), ×8000 (*b*).

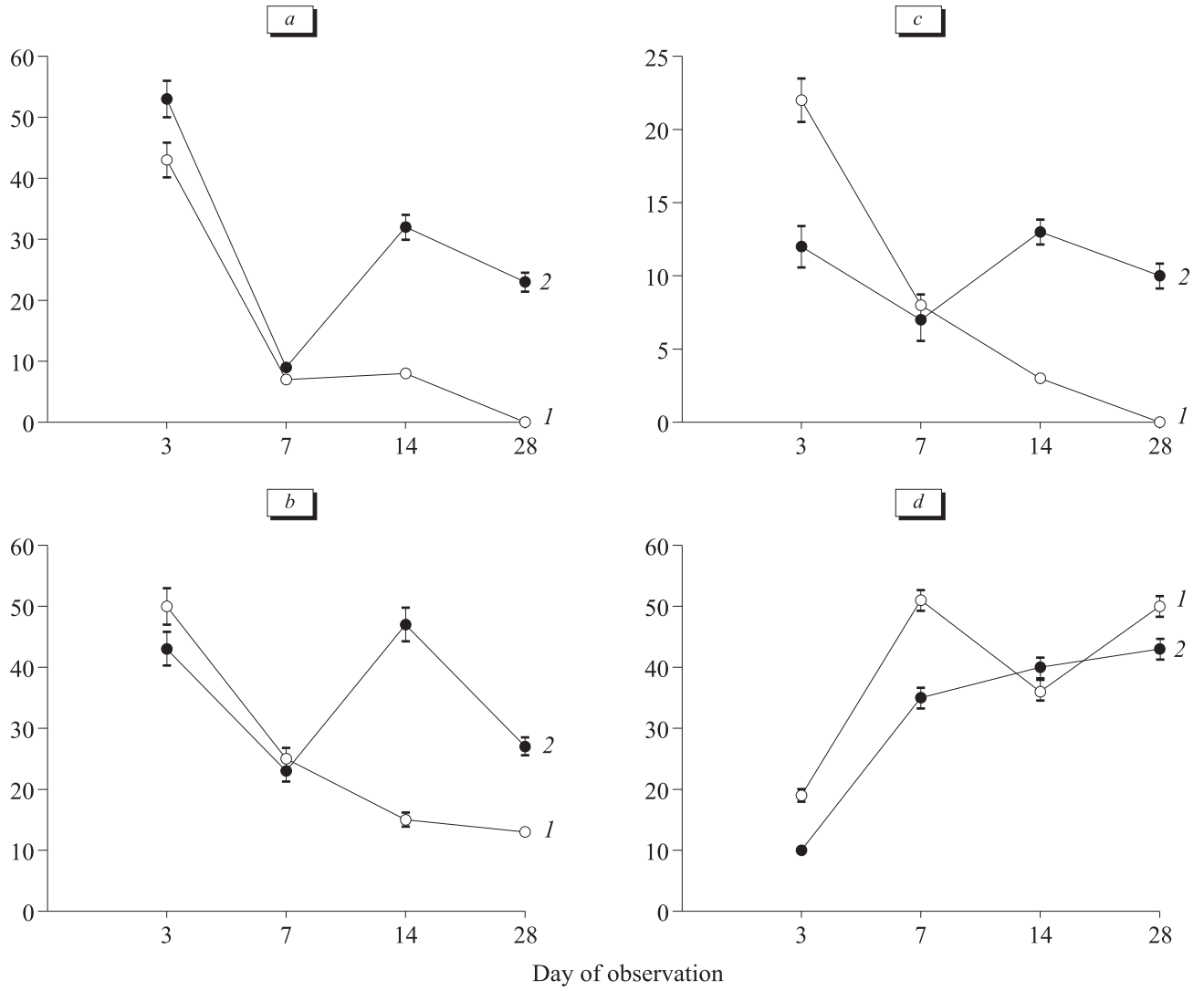

Fig. 3. Dynamics of cellular composition at the site of Prolen (*1*) and Esfil (*2*) implantation. *a*) neutrophilic granulocytes; *b*) macrophages; *c*) lymphocytes; *d*) fibroblasts. Ordinate: number of cells in 10 fields.

2, *d*), while in cases with Prolene the number of cells was negligible.

By day 28 cellular reaction was presented mainly by fibroblasts. Horizontally oriented spindle-shaped mature fibroblasts (collagenoblasts) predominated among synthesized collagen fibers in tissue. Their nuclei were characterized by accumulation of heterochromatin along the caryolemma and as lumps inside it. Granular endoplasmic reticulum was well developed and presented by cisterns filling virtually the entire cytoplasm, the mitochondria had clarified matrix and partially retained cristae. Accumulation of lipid granules, characteristic of functionally active fibroblasts, was seen at the cell periphery [3]. Numerous collagen structures of different degree of maturity (from scanty fine slightly striated fibrils to large striated collagen bundles) were seen around these cells; Fig. 2, *e*). Collagen fibers formed a wide connective tissue capsule around the endoprosthesis. Connective tissue components totally grew into the implant. Devastation of vessels, slight macrophagic infiltration were observed.

We singled out some morphological differences in tissue reaction to implantation of the endoprostheses. More pronounced infiltration by neutrophilic granulocytes, macrophages, and lymphocytes was observed 2-4 weeks after implantation of Esfil net. These differences in cellular reactions can be due to physical characteristics and pattern of the network. Prolen net consists of nonresolvable fibers made from isotactic crystal stereoisomer polypropylene, synthetic linear polyolefine $(C_3H_6)_n$, identical by composition to the material from which Prolen suturing material is made. The net is about 0.6 mm thick, the diameter of the thread is 0.15 mm, surface density 95.9 g/m^2, pore size 0.56-0.63 mm.

The network provides fixation in all sites of fiber connection and can be stretched in both directions. Fiber connections endure no lesser load than the fibers of more rigid metal nets. Due to elasticity of the net it adapts to loading. Prolen net is highly resistant to rupture (about 14 kg/cm^2) and stretching. Esfil net is made from biologically inert polypropylene monothread, it is not resolved and does not loose its strength and elasticity under the effect of tissue fluids. Special network pattern provides high strength and stability of size to the reticular endoprosthesis; its edges are not loosened when cut, its thickness is 0.47 mm, thread diameter 0.10 mm, surface density 53.0 g/m^2.

According to published data [9], implantation of Prolen polypropylene net causes the development of an acute inflammatory reaction at the site of endoprosthesis. One week after the operation connective tissue grew into the net, which was paralleled by pronounced infiltration by polymorphonuclear granulocytes and macrophages. Later the count of granulocytes gradually decreased, while that of macrophages increased, and 3 months after implantation they became the predominant population. Macrophage transformation into epithelioid cells and foreign body-type cells near the implant fibers was observed. By day 21 the number of macrophages and epithelioid cells markedly decreased, while that of granulocytes remained high. Electron microscopy showed apoptotic macrophages. The inflammatory reaction remained acute with few polymorphonuclear granulocytes and formation of epithelioid granulomas with foreign body-type giant cells up to 3 months after implantation. The degree of connective tissue maturation with the formation of well vascularized connective tissue capsule around the implant increased starting from day 14 and peaked by day 28 postoperation [4]. Implantation of Russian-made endoprostheses, *e. g.* Esfil net, caused no pronounced tissue reaction. Despite inflammatory reaction at early terms, close by its intensity to that after implantation of Prolen endoprosthesis, implantation of Esfil eventuated in the formation of a compact fibrous connective tissue capsule, reliably insulating the implant.

Hence, both endoprostheses (Prolen and Esfil) well integrate in the adjacent tissues in the implantation zone and can be recommended for clinical use. Implantation of Esfil net is associated with more pronounced cellular reaction. The formation of connective tissue capsule around the endoprostheses is observed at the same time. A characteristic feature in both cases is the absence of the foreign body giant multinuclear cells.

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