ORIGINAL PAPER

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

Experimental study regarding the biocompatibility test of the Prolene (polypropylene abdominal mesh) product

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Abstract

The polypropylene mesh, although is one of the most used prosthetic biomaterials for abdominal wall defects, proved not to be completely inert, generating from precocious foreign body inflammatory reactions (varying by individual reactivity, the amount of used material and its structure), to late complications such as chronic infections, stercoral fistulae or mesh migration. The present paper was aimed at studying the behavior of implants of this material in three different areas of the body of experimental animals, as follows: intramuscular, intraperitoneal and extraperitoneal. The observation time was 21 days and 90 days. We observed foreign body reactions induced locally by the mesh that remains temporary, generating a moderate number of macrophages and foreign body giant cells. The material did not systemically affect the healing and the scaring of the surgical wounds, but in all three implant areas, the polypropylene mesh generated locally a fibrous proliferation reaction of neoformation tissue, which wrapped and secured the implanted product on all surfaces.

Keywords: biocompatibility, extraperitoneal, intramuscular, intraperitoneal, mesh.

Introduction

Incisional hernias have an incidence ranging between 9–20% [1–5]. At least one in 10 patients who undergoes median laparotomy develops an incisional ventral hernia. One-third of these patients develop complications such as intestinal obstruction, strangulation, which require emergency surgical intervention [6–10]. Approximately 31% up to 55% [11, 12] of anatomical procedures as a treatment for parietal defects result in relapse, as for alloplastic procedures was observed that the positioning of the polypropylene mesh in direct contact with the intraperitoneal organs is not recommended, causing intervisceral adhesions and fistulas in 80–90% of the patients [13, 14].

The polypropylene mesh is one of the most common prosthetic biomaterials used for abdominal wall defects in humans [15]. The mesh was introduced in 1958 by Usher *et al.*, being subsequently popularized by Lichtenstein [16–20]. This material proved not to be completely inert, causing sometimes foreign body inflammatory reactions, depending on the amount of used material and its structure. Subsequently, late complications have been reported, for example chronic infections, stercoral fistulae or mesh migration. Although pathogenetic mechanisms involved in these phenomena are poorly understood, they are definitely influenced by the inflammatory cascade induced by the mesh in the host organism. A moderate inflammation induced by the bioderivative matrix makes it more biocompatible [13, 21].

Aim

The experiment aimed at studying the behavior of fragments of the polypropylene mesh in three different areas of the body, as follows: intramuscular, in the thigh muscles; intraperitoneal; extraperitoneal, replacing a segment of the abdominal wall.

Materials and Methods

To this end were made three batches of experimental animals that are suitable for this study, Wistar rats, adult males weighing 180 g, each batch containing a total of 10 individuals.

Time of observation of the subjects after the surgery was 21 days, respectively 90 days, according to ISO rules.

The material to be tested was sectioned, each fragment having an approximate dimension of 1 cm^2, sterilized in advance (Figure 1).

Implantation was performed by surgical act after general anesthesia with a cocktail consisting of Vetased – 10% solution in the amount of 80 mg/kg body weight (bw) 2 p and Xylazine Bio – 2% solution in the amount of 8 mg/kg bw 1 p, 0.3 mL intramuscularly administered.

After introduction of implant according to the above-described procedure (Figures 2–4), the wound was sutured with absorbable thread, the subjects being consequently maintained under standard life conditions and being subjected to observation daily until the end of the experiment.

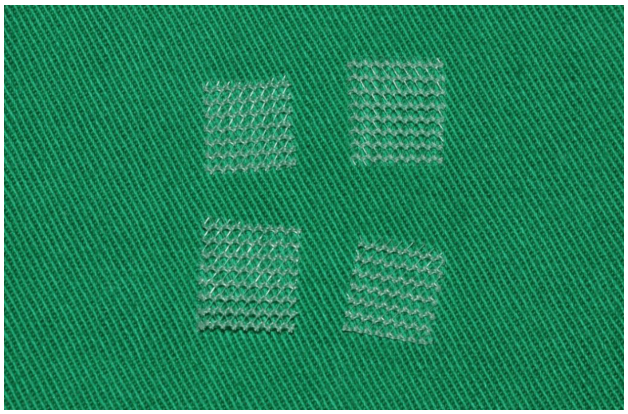

Figure 1 – ***Structural features and mesh configuration.***

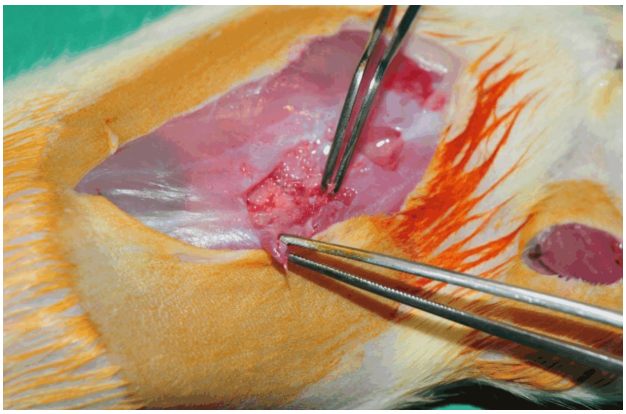

Figure 2 – ***Intramuscular positioning of the mesh.***

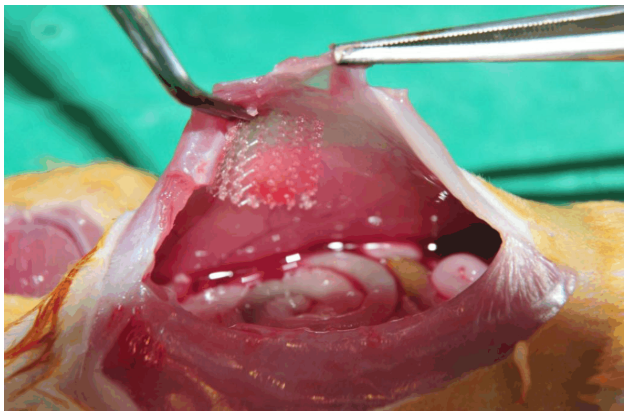

Figure 3 – ***Intraperitoneal positioning of the mesh.***

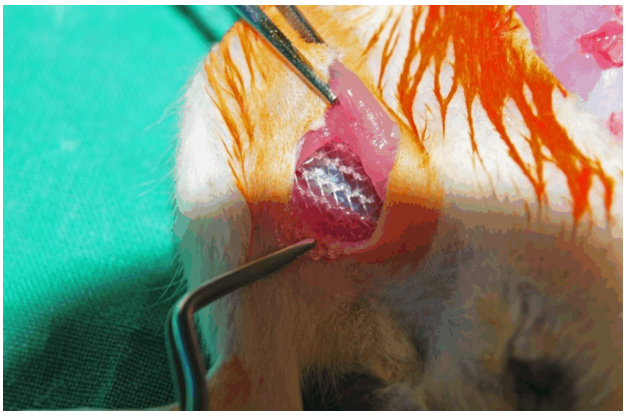

Figure 4 – ***Extraperitoneal positioning of the mesh.***

After 21 days, five individuals from each group subjected to the experiment were euthanized and was proceeded to the examination of the tissues adjacent to the implant. From each animal, tissue was harvested and macroscopic and histopathological examinations were made. Histopathological exam was performed on portions of muscle tissue, the parietal peritoneum and visceral peritoneum from the contact areas with the implant.

Ninety days after the implantantion, we proceeded to examine the other animals in each group. The objectives of the evaluation were the state of the implant, the host tissues reaction and the produced histopathological changes.

After the macroscopic examination of the meshes, at the two time intervals, samples for histological examination were collected, to reveal the tissue integration and the local behavior of the meshes. The biomaterial, the abdominal fascia and the peritoneum were harvested in bulk from the junction with the host tissue.

For the histopathological exam, the collected tissue samples were placed in fixation agent (10% buffered formalin, pH 7) for 24 hours. Samples were processed by paraffin-embedding technique. Sections were made at 4.5 μm with the Leica RM 2125 RT microtome and then stained with Hematoxylin–Eosin (HE) for better differentiation of cell types and with Masson's trichrome (MT), which allows observation in a selective manner in chromatic terms of the muscle fibers, fibrin, cellular elements and particularly the collagen fibers. Subsequently, the samples were examined under an Olympus BX 51 microscope. The images were captured using an Olympus DP digital camera and processed using the Olympus Cell B image acquisition and process program.

Results

After 21 days

After the implant both wounds in the thigh and the abdomen were closed and healed *per primam*, after a period of 5–7 days. At 21 days the wounds were healed, observing only a whitish line, following a *per primam* scar (Figure 5). Throughout all the postoperative period, there were no observed changes in the general condition of the subjects, surpassing quickly and easily the convalescence period. Throughout the 21-day observation, on the animals in all groups, no significant changes were noted in the

implant area and they did not show phenomena of rejection or infection.

Examination after 21 days of the tissues brought in contact with the implanted material revealed in the muscle (thigh area) that the insertion of the implant fragment generated an intermuscular (interstitial) connective tissue local reaction, which embedded the implant on all sides, fitting it between the muscle bundles.

Intraperitoneal insertion of the mesh segment caused a reaction of both the parietal peritoneum and the omentum. The material fragment is secured by a connective proliferation, which encompasses the fragment on all sides. The fibrous connective proliferative process at the same fixes the implant to the parietal peritoneum or the epiploon by limited areas of adhesion (Figure 6).

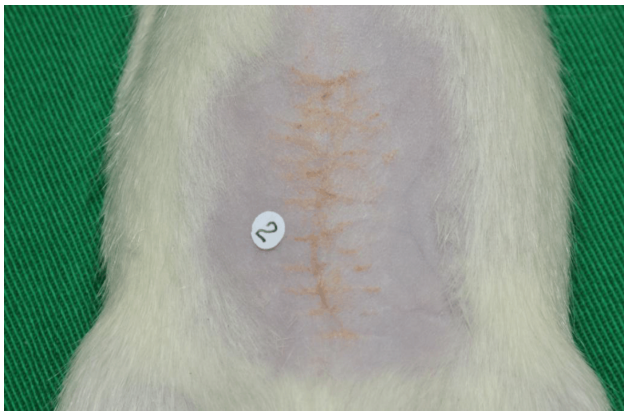

Figure 5 – ***Intraperitoneal positioning. Healed surgical incision in the lower limb – healed per primam at 21 days postoperatively.***

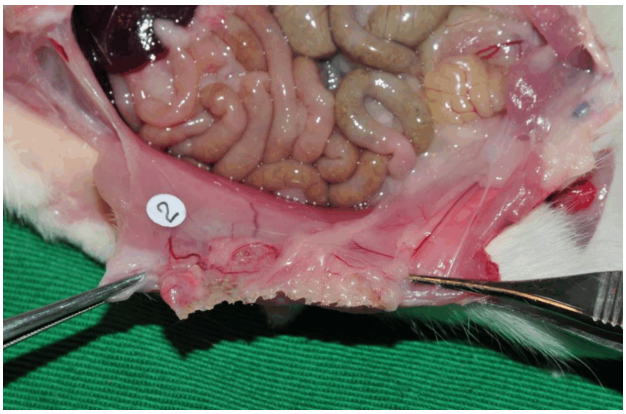

Figure 6 – ***Fibroplasia and connective penetration phenomena surrounding the implant (mesh).***

In the case of the extraperitoneal insertion of the mesh segment, after 21 days from the implant was observed that the abdominal wall was healed *per primam*, without inflammatory rejection reaction. The macroscopic examination of the extraperitoneal implant revealed a normal reaction of the parietal and visceral peritoneum, without adhesions. The implant fragment is secured by a connective proliferation, which embedded it perfectly.

The presence of the implant fragments thus causes in the host tissue (muscle and peritoneum) a response from the interstitial connective tissue. It proliferates a neoformation tissue, which infiltrates through the mesh holes wrapping each strand in a connective tissue collar.

After 90 days

Like the results after 21 days, the observation made after 90 days in both thigh area (intramuscular) and abdominal wall (intraperitoneal and extraperitoneal implant), the wounds were healed. At the end of the experiment (90 days), there was basically just a white line, following an uncomplicated scar (Figure 7). Tissues are well strengthened, which shows that the inserted material is well tolerated by the adjacent tissues.

In the muscle tissue the same local reaction of the intermuscular connective tissue occurs, which embedded the implant segment even better (Figure 8).

The intraperitoneal reaction produced by the inserted segment, after a period of 90 days, is an active connective proliferation of the adjacent tissues that embedded on all sides the foreign material (Figure 9).

The macroscopic examination of the subjects' body reaction, 90 days after the extraperitoneal implant, reveals that the abdominal wall has perfectly healed and strengthened (Figure 10).

The examination of the abdominal wall and peritoneal tissues put into contact with the inserted material reveals a proliferative fibrous connective process, which at the same time secures the inserted segment with the surrounding tissues and strengthens the abdominal wall.

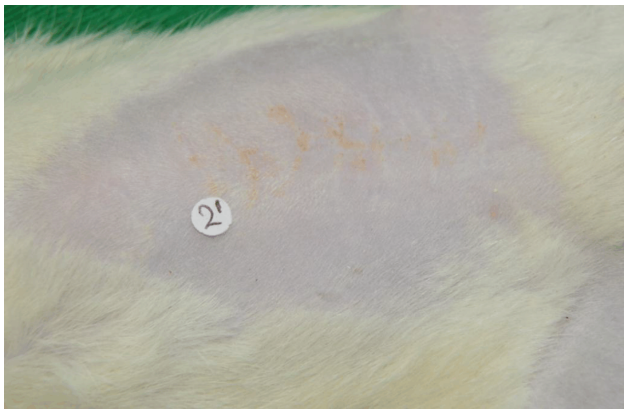

Figure 7 – ***Intraperitoneal positioning. Surgical incision healed per primam.***

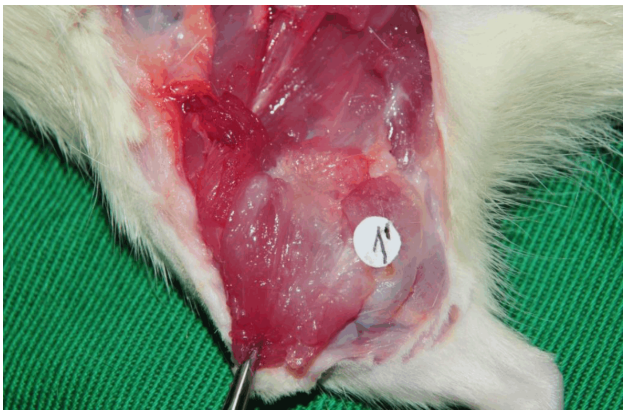

Figure 8 – ***Fibroplasia and conjunctive penetration phenomena; intramuscular implantation of the product.***

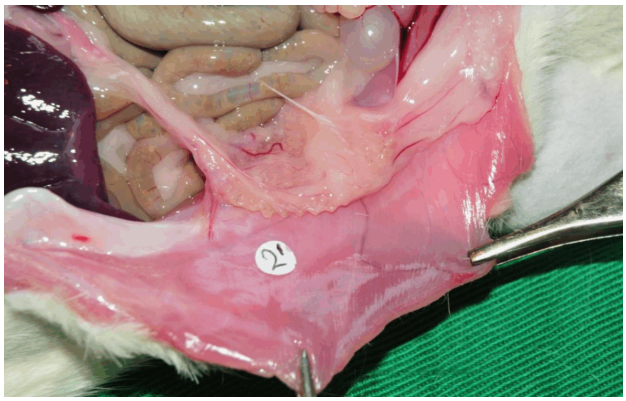

Figure 9 – ***Fibroplasia and connective penetration phenomena incorporating the mesh.***

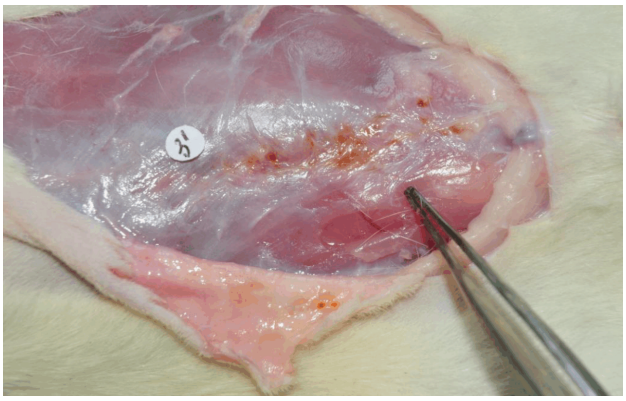

Figure 10 – ***Perfect healing of the abdominal wall, 90 days after surgery.***

Histological assessments

At the histological assessment, all the implants were well integrated into the autologous tissue, being surrounded by a dense granulation tissue, with numerous collagen fibers arranged circularly around the threads of the net. On the edge of the net, in the granulation tissue, there were skeletal muscle cells with atrophy or necrosis and macrophage resorption.

Twenty-one days after the implant, there was a chronic inflammatory reaction with fibrous connective proliferation and macrophage inflammatory infiltrate, ordered both perifilamentary and interfilamentary. Around the meshes was observed formation of inert foreign body granulomas. Fibroplasia around the implant is intense, this connective tissue having a bilayer aspect: a loose periphery area and a dense area around the implant. The loose area has numerous microvessels and the collagen fibers are fewer (Figures 11–16).

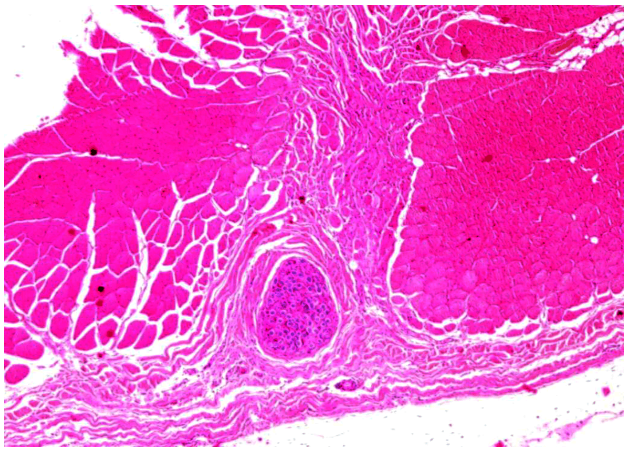

Figure 11 – ***Intraperitoneal positioning 21 days after surgery. Fibroplasia and connective penetration phenomena around the implant. HE staining, ×40.***

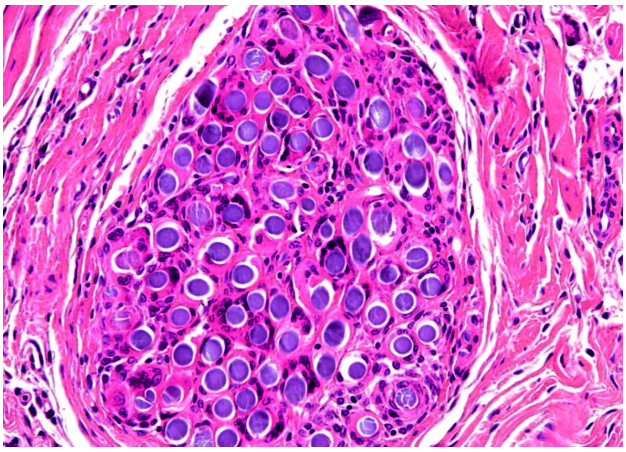

Figure 12 – ***Intraperitoneal positioning 21 days post-operative. Fibroplasia and connective penetration phenomena around the mesh; connective tissue in between the mesh monofilaments. HE staining, ×200.***

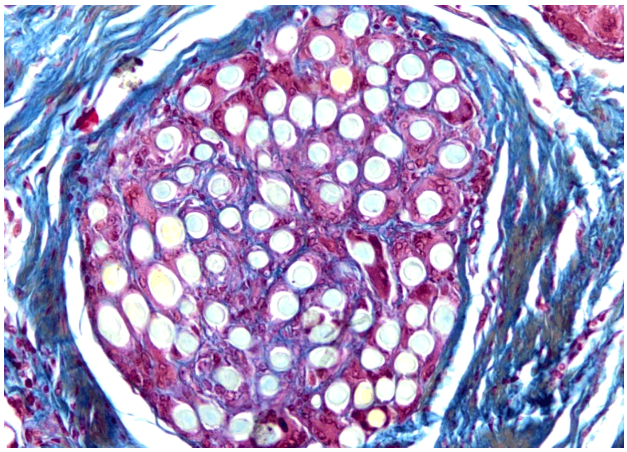

Figure 13 – ***Intraperitoneal implant after 21 days. Fibroplasia and connective penetration phenomena surrounding the implant, mast cells and giant cells in between the meshes microfibers. MT staining, ×200.***

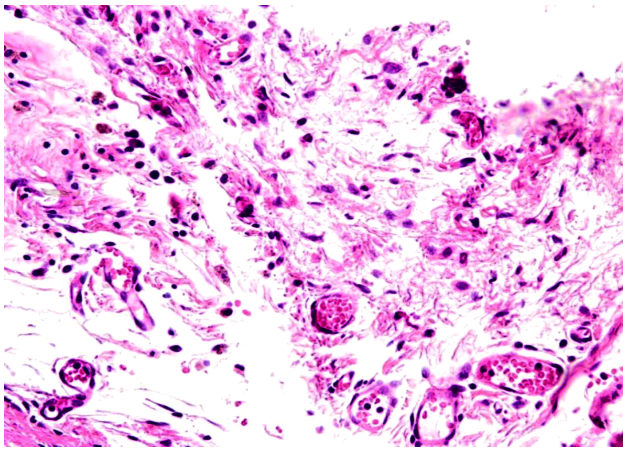

Figure 14 – ***Extraperitoneal positioning of the mesh. Lax connective tissue, well vascularized, involving mast cells and rarely neutrophil cells. Mast cells filled with ceroids. HE staining, ×200.***

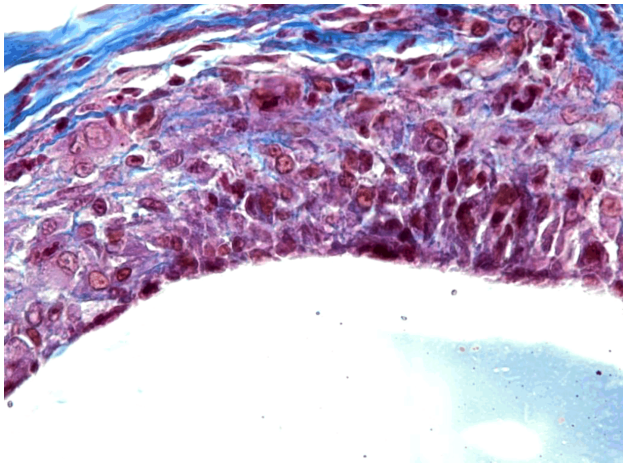

Figure 15 – ***Extraperitoneal positioning 21 days after surgery. Giant epithelioid reaction around the mesh. MT staining, ×400.***

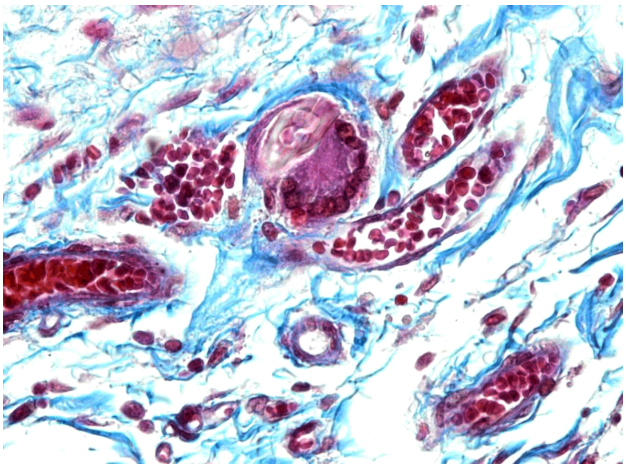

Figure 16 – ***Extraperitoneal implant 21 days after surgery. Mesh monofilament, giant cell, lax fibrous tissue, well vascularized. MT staining, ×400.***

Ninety days after the implant, histopathological examination revealed the same proliferative reaction of a strongly vascularized neoformation tissue, inert foreign body granulomas formation with numerous macrophages and giant cells, as the same as the 21 days period. The difference between the reaction after 21 days and the one after 90 days is the intensity of the fibrosis as well as the macrophage resorption processes, which are more obvious after 90 days (Figures 17–19).

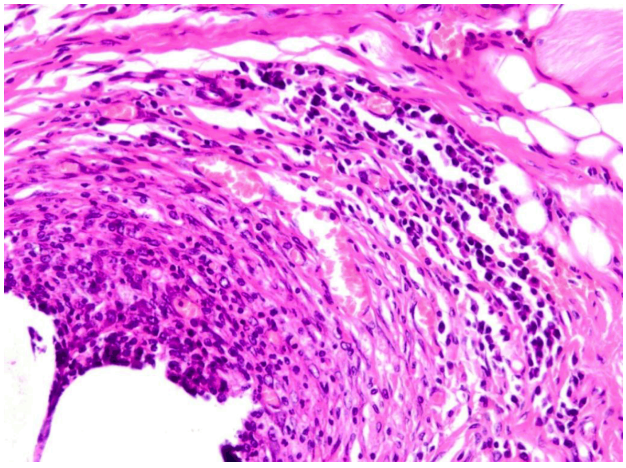

Figure 17 – ***Intraperitoneal implant after 90 days. Intense fibro-connective reaction around the implant, abundant inflammatory infiltrate dominated by lymphohistiocytes. HE staining, ×200.***

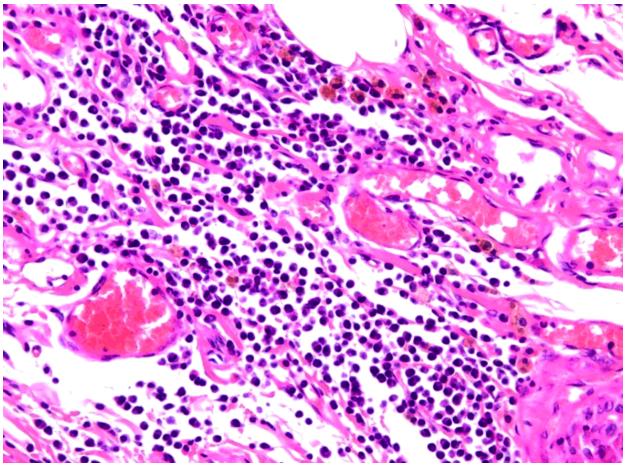

Figure 18 – ***Intraperitoneal implant 90 days after surgery. Fibrosis, neovessels, abundant infiltrate with mast cells. Mast cells filled with ceroids. HE staining, ×200.***

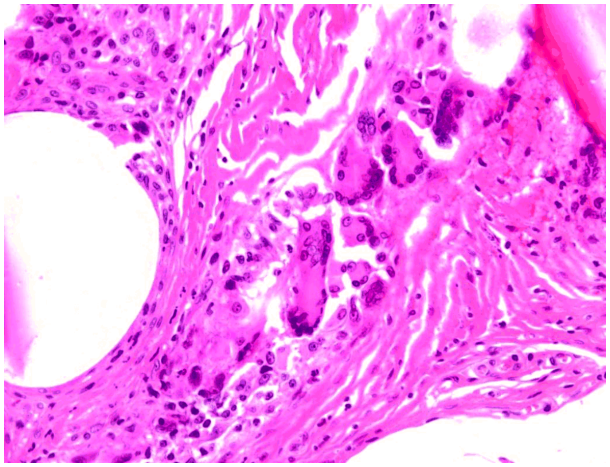

Figure 19 – ***Extraperitoneal implant 90 days after surgery. Foreign body granuloma, giant cells. HE staining, ×200.***

Discussion

In the last three decades, many types of prosthetic biomaterials have been tested. There are still many controversies on the ideal implantation design [16, 22–24]. The critical component in the mesh architecture is the porosity, tissue incorporation being direct proportional to the degree of porosity. Macroporous meshes allow fibrous tissue penetration and incorporation into the host tissue. Polypropylene falls into this category of biomaterials, being macroporous. The poor surface roughness of polypropylenic fibers and the blending texture stimulate the fibroplasia and conjunctive penetration phenomena.

Fibrous tissue was less dense in the first period, with cellular dominance and irregularly developed collagen fibers. In both time intervals, it is observed the persistence of the monocytes and foreign body giant cells, with no significant differences between fixation types.

From the surgical point of view, the inserted biomaterial must allow a good interfilamentary and perifilamentary tissue growth, to ensure adequate strength of the abdominal wall [25–28], polypropylene proving effective in this regard.

The polypropylene mesh may induce foreign body reactions [29–32]. After the insertion into the host tissues, the implants did not show acute effects of rejection and the material did not affect the healing or the scaring process of the surgical wounds used for introduction of the intramuscular, intraperitoneal or extraperitoneal implant.

All fixations ensure a good penetration of the fibrous tissue and the collagen matrix, maintaining a chronic foreign body reaction with moderate number of macrophages and foreign body giant cells [33, 34].

Neovascularization phenomena are present in both postoperative time periods and gradually increase both interfilamentary and perifilamentary.

It is important to observe in time the phenomenon of tissue integration of the meshes, thus revealing the long-term effects of such an implant. The material subjected to the test behaved similar in all three implant areas (intramuscular, intraperitoneal and extraperitoneal), both after 21 days and 90 days, which is consistent with other specialized studies [33].

The implant did not produce rejection effects by the host organism, thus the material has not influenced the healing and scaring of the surgical wounds, observation supported by the study of the specialized literature [35].

In all three implant areas the material locally determined a neoformation tissue fibrous connective proliferation reaction, which wrapped and secured on all surfaces the implanted product [36], as seen in the literature data [35, 37–40]. The connective tissue developed around portions of the mesh is a newly formed tissue consisting of collagen fibers arranged circularly around each strand of the structure. It creates neoformation capillaries and a massive reaction of fibroblasts, monocytes, macrophages and foreign body giant cells [41–43].

Conclusions

After the implantation of the polypropylene material in the three different areas of the experiment animals, intramuscular (in the thigh muscles), intraperitoneal, extraperitoneal (replacing a segment of the abdominal wall), macroscopically after 21 days there were no changes reported in the implantation area and there were no phenomena of rejection or infection. After 90 days, the alloplastic material was well integrated and consolidated. Microscopically, after 21 days it was observed a chronic inflammatory reaction with fibrous connective proliferation and macrophage inflammatory infiltrate, ordered both perifilamentary and interfilamentary. The connective tissue around the implant has a bilayer aspect: a loose area in the periphery with numerous microvessels and fewer collagen fibers, respectively a more dense area around the implant. After 90 days, it was revealed a high-vascularized neoformation tissue, with numerous macrophages and giant cells. The histological difference between the two time periods is represented by the intensity of the fibrosis and the growth of the macrophage resorption processes, that are more obvious in a longer time period. Unfortunately, there is no follow-up of the implantation areas of the alloplastic material on the experience animals with a length of time longer than 90 days.

Conflict of interests

The authors declare that they have no conflict of interests.

Author contribution

All authors contributed equally to the manuscript.

Acknowledgments

This paper is financed from the European Social Fund and by the Romanian Government under the contract number POSDRU/159/1.5/S/137390.

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Received: September 29, 2015

Accepted: April 28, 2016