The Response of the Local Immune System to the Introduction of Polypropylene Synthetic Implants (Experimental Study)

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Abstract
The number of operations increases together with the number of implant-associated complications despite the good biocompatibility and inertness of modern mesh implants. The pathogenesis of these complications is important to be investigated in the conditions of the experiment. This study aims at investigating the peculiarities of tissue reactions in the experiment with introduction of a suspension from polypropylene mesh implant into the abdomen with minimal surgical trauma. The fragment of the implant frozen in the cryostat was cut with a microtome to particles smaller than 10 μm, then dried, sterilized, resuspended in sterile saline, and injected intraperitoneally to small laboratory mice with a syringe. The parameters of the functional activity of various biologically active substances were further examined to estimate the reactions of the experimental animals to the introduction of a mesh implant. The animals were withdrawn from the experiment after 20, 40, and 60 days. On the 20th day after the suspension introduction, the phagocytic index significantly decreased by 25%. The values of IL-1 and IL-5 in the lavage samples did not exceed the sensitivity of the used method (25 pg/ml). Elevated levels of IL-4, IL-6, IL-22 and interferon-γ were found in the lavage of only few animals. Thus, a good biocompatibility and inertness of the synthetic material have been confirmed. A moderate local immunosuppressive effect of the implant suggests that any deviations in the more traumatic surgical manipulations than in the experiment will increase the risk of complications. In addition, the degree of local immunosuppression depends on the tissue condition at the time of surgery. Moreover, the method of the exudate extraction at any stage of the postoperative period can be used for other purposes.

Keywords: Mesh Implants; Tissue Reaction; Biocompatibility; Experimental Animals

Introduction
The widespread use of synthetic materials in surgery to strengthen tissues began in the mid-20th century. Moreover, both in general surgery (for hernioplasty) and in narrower specialties, such as obstetrics and gynecology [1,2]. The criteria for “ideal” synthetic plastic materials were formulated back in 1950 and have not changed much until now [3]. These materials should not change their physical properties under the influence of physiological environments; must be chemically inert; should not cause a pronounced inflammatory reaction during implantation; should not cause scarring, etc. In 1994, P.K. Amid., et al. added two more important characteristics: the ability to germinate with their own tissues and the simplicity of implant placement [3].

It must be emphasized that modern mesh implants (SI) used in gynecological and surgical reconstructive operations have all the specified parameters. Despite this, in parallel with the increase in the number of operations performed, the number of so-called “implant-associated” or otherwise “mesh-associated” complications (IAO), such as, for example, erosion of the mucous membrane of the vaginal wall, also grows [4,5]. The average frequency of erosion after such operations in gynecology is according to different authors 7.6 - 10.6% [6,7].

In this regard, it seems important in the conditions of the experiment to clarify what exactly the complications are associated with. It should be borne in mind that the existing objective difficulties in placing SI in the pelvic floor in experimental animals significantly limit the ability to simulate surgery in an experiment.

The restrictions include: the inability to use small rodents (mice, rats, guinea pigs); technical difficulties (small size of the surgical field, lack of specialized surgical instruments for operations on medium experimental animals (rabbits, sheep, macaques). In this regard, the design of the study often has to be changed.

To evaluate the biocompatibility of SI, various experimental methods have been proposed [8]. In particular, in experiments on rabbits, it was previously shown that when polypropylene and porcine collagen implants were inserted into the posterior vaginal wall, a moderate inflammatory reaction with minimal fibrosis occurred in both cases [9]. To study the process of adhesion formation, synthetic SI were implanted into the abdominal cavity of mice [10]. An implant made of a polypropylene mesh induced the appearance of a pro-inflammatory medium, however, the inflammatory reaction was short-term (about 2 weeks) and did not provoke adhesions.

Very important indicators reflecting the biocompatibility of the material are indicators of the phagocytosis system and the levels of pro-inflammatory and anti-inflammatory cytokines in tissues and exudate from the recipient zone. It is quite clear that it is not possible to cumulate (and subsequently examine) vaginal exudate in laboratory animals. In this regard, other laboratory systems are needed.

Objective of the Study

To study the characteristics of tissue reactions under experimental conditions when a suspension of a synthetic mesh implant based on polypropylene is introduced into the abdominal cavity with minimal surgical trauma.

Materials and Methods

For the introduction into the abdominal cavity of experimental animals, the following sample preparation technique was used. Fragments of the GynoMesh PS macroporous polypropylene mesh (Ethicon, J&J, USA) used earlier in the Prolift, Prosima sets, and now in the TVT, TVT-Obturator and other sets, were crushed and then fixed with distilled water on a frozen aluminum block in a Leica CM 15105 cryostat at a temperature of minus 19°C. After fixing the polypropylene mesh in ice, numerous sections of the preparation were made with a section thickness of 5 μm. Then, the resulting heterogeneous mixture was collected in a Petri dish and transferred to a thermostat, where it was kept at a temperature of 27 - 31°C for 6 hours. After evaporation of water, a dry residue was formed in the form of a “powder” from a crushed polypropylene mesh, without any impurities. Next, the Petri dish with the ground sample was sealed in a sterilization bag and sterilized with 58% hydrogen peroxide using a Sterrad NX sterilizer in a normal cycle at a temperature of 40°C for 28 minutes.

The study was performed on female CBA × C57BL/6 hybrid mice (Rappolovo Laboratory Animal Nursery). The animals were kept in the vivarium of the FSUE “GosNII OCHB” FMBA of Russia under mixed lighting, received drinking water and granulated ad libitum food. The crushed powder of the polypropylene mesh was resuspended in sterile physiological saline and the mice of the experimental group (30 animals) were injected intraperitoneally with 2 ml of suspension per animal using a 18G syringe. Control animals (30 animals) received an injection of a similar volume of saline.

The choice of the place for the introduction of the studied material was based on the phenomenon proved back in the 80s of the 20th century that the biocompatibility of implants, regardless of their location, is controlled, first of all, by the macrophage component of the tissue

reaction [11]. Moreover, for the study of the in vitro interaction of cells and biomaterials, the choice of the cell type is crucial. In vivo data show that during the healing of an implant in tissues, macrophages are the main type of cells that determine the nature of healing [12].

Another very important advantage is that it is relatively easy to get lavage fluid from the abdominal cavity for research.

In order to minimize surgical trauma, an injection technique was proposed for introducing the test material into the abdominal cavity. Accordingly, for this it was necessary to obtain samples of polypropylene in the form of a suspension, which was achieved using special technology (patent registration number 2015116545). Sterilization of the resulting suspension excluded the involvement of a bacterial component in the inflammatory reaction. The crushed and sterilized polypropylene implant retains its physicochemical properties, since during the preparation process it was not exposed to high temperature, aggressive abrasive or chemically active agents. Microscopic data confirmed the absence of surface changes on the sterile fragments of the crushed implant.

Animals were removed from the experiment after 20, 40 and 60 days. After cervical dislocation, the animals were washed with the abdominal cavity with 5 ml of cold RPMI-1640 medium (Bilot LLC) for 2 minutes. The concentration of cells in lavage fluid was counted in the Goryaev chamber, the required volume was selected to evaluate phagocytosis of peritoneal macrophages. The remainder of lavage fluid was centrifuged for 10 minutes at 200g, a precipitate was taken and frozen at minus 20°C for subsequent determination of cytokine levels. After lavage, the abdominal cavity of the animals was opened and examined for macroscopic changes.

When evaluating the phagocytic activity of peritoneal macrophages, baker’s yeast opsonized with mouse serum was used as an object of phagocytosis. After taking peritoneal lavages and counting the cellularity, a lavage volume containing 1 x 10⁶ cells was taken, the volume was adjusted to 2 ml with RPMI-1640 medium with 10% fetal calf serum (Bilot LLC), and the obtained cell suspension was layered onto 40 mm diameter Petri dishes (Medpolymer). The plates were incubated at 37°C for 1 hour to adhere macrophages to the plastic. Then the medium from the plates was removed, neatly adhering cells were washed gently with warm medium. Then 1.8 ml of fresh medium with 10% serum and 0.2 ml of suspension of opsonized yeast were added to the cups. After this, the plates were re-incubated at 37°C for 1 hour and then washed from non-phagocytosed yeast with saline. Then, physiological saline was removed, the preparations were dried in cups in air, fixed with 96% ethanol and stained according to Romanovsky-Giemsa.

The calculation was carried out at a magnification of 160x under immersion. The intensity of phagocytosis was characterized using the phagocytic number (PS) - the percentage of macrophages that entered into phagocytosis, and the phagocytic index (PI) - the average number of yeast phagocytosed by one macrophage. The significance of differences between the groups was evaluated using an unpaired student test with unequal deviations. Differences were considered significant at p < 0.05.

In the obtained samples of peritoneal lavages of experimental animals, the concentrations of the following cytokines (interleukin (IL) -1; IL-4; IL-5; IL-6; IL-10; IL-13; IL-22 and interferon-γ) were determined using commercial Bender MedSystems FlowCytomix kits according to the manufacturer’s instructions on an EPICSXL flow cytometer (BeckmanCoulter). Analysis of the results (construction of calibration curves and calculation of cytokine concentrations in the studied samples) was performed using the FlowCytomix Pro2.3 program (Bender MedSystems). Next, the mean and standard deviations of the concentrations of the studied cytokines in the control and experimental groups of animals were calculated, the significance of differences was evaluated using an unpaired Student’s T-test with unequal deviations.

Results and Discussion
To assess phagocytic activity, a phagocytic number (PS) was used, i.e. average number of particles absorbed by one phagocyte and phagocytic index (PI), i.e. the ratio of the number of phagocytes absorbing particles to the total number of phagocytes viewed [13].

In the course of the study of the phagocytic function of mouse peritoneal macrophages after the introduction of the crushed polypropylene network, the following was established (Table). On the 20th day after the introduction of the suspension containing the crushed

polypropylene mesh, a significant decrease in the phagocytic index by 25% was recorded. However, in subsequent time periods, differences between the groups were not recorded.

<table>
<thead>
<tr>
<th>Day after administration of the suspension</th>
<th>PS,%</th>
<th>FI, conv. units.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>The control</td>
<td>Experience</td>
</tr>
<tr>
<td></td>
<td>95.1 ± 2.3</td>
<td>95.0 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>97.2 ± 1.4</td>
<td>95.0 ± 4.1</td>
</tr>
<tr>
<td></td>
<td>97.3 ± 1.8</td>
<td>93.7 ± 4.4</td>
</tr>
<tr>
<td></td>
<td>12.1 ± 0.8</td>
<td>8.9 ± 1.2*</td>
</tr>
<tr>
<td></td>
<td>12.6 ± 2.5</td>
<td>14.8 ± 2.8</td>
</tr>
<tr>
<td></td>
<td>15.2 ± 1.0</td>
<td>15.5 ± 2.5</td>
</tr>
</tbody>
</table>

Table: Dynamics of phagocytosis of peritoneal macrophages of experimental animals.
* <0.05 compared with the control group.

When studying the parameters of the cytokine reaction, the following was established. In relation to IL-1 and IL-5, in no lavage samples were found values exceeding the sensitivity of the method used (25 pg/ml). Elevated levels of IL-4, IL-6, IL-22 and interferon-γ were found in lavages only in single animals. Minimum concentrations of IL-10 (from 2 to 15 pg/ml) and IL-13 (from 2 to 25 pg/ml) were determined in peritoneal lavages in about half of the animals, with no significant differences between the control and experimental groups.

The results of the study demonstrate that our proposed technique, namely, that the implant fragment frozen in a cryostat is crushed using a microtome to particles smaller than 10 μm in size, dried, sterilized, resuspended in sterile saline and injected intraperitoneally into small laboratory animals using a syringe and further investigate the indicators of the functional activity of various biologically active substances allows us to reliably evaluate the reaction of the organism of an experimental animal to e SI. At the same time, the ability to carry out the sampling of the resulting exudate at any stage of the postoperative period can be used for other purposes and tasks.

The proposed method implements a new experimental approach to assessing the biocompatibility of mesh synthetic implants, provides minimal surgical trauma, the ability to study cellular and cytokine reactions of local immunity, allows the use of small laboratory animals, which simplifies and reduces the cost of the experiment, enhances the capabilities of researchers.

During the experiment, it was found that with a decrease in surgical trauma by injecting a suspension of the ground polypropylene network GyneMesh PS, after the completion of the acute reaction of the body to a foreign body (20 days), a moderate local immunosuppressive effect of the studied foreign body continues, manifested in a decrease in the phagocytic activity of peritoneal macrophages. At later dates (40 and 60 days), the studied parameters in the experimental animals did not differ from the control.

Conclusion

Thus, rather good biocompatibility and inertness of the synthetic material used in the Prolift, Prosima, TVT, TVT-Obturator, etc. kits allows minimizing the likelihood of IAO. On the other hand, a moderate local immunosuppressive effect of SI suggests that any deviations during more traumatic surgical procedures than under experimental conditions will increase this risk. In addition, it seems reasonable to assume that the degree of local immunosuppressive effect detected by SI can depend on the characteristics of the state of the tissues at the time of surgery.

Bibliography

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