

CLINICAL RESEARCH

## Silver Nanocluster Reparative Effect in Hernioplasty

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### Abstract

**Background:** The acceleration of re-epithelialization and fibroblast differentiation were noted during the experiments with silver nanoclusters (SNs) by interrupting the negative development of inflammation at the level of cytokines and promoting a positive course of reparative processes. **The aim** of this work was to elaborate the experimental model of prosthesis hernioplasty in subcutaneous and intraperitoneal locations of hernioprotheses with SNs, which allowed us to study the course of reparative reactions in all layers of the anterior abdominal wall.

**Material and Methods:** We used a modified hernioprosthesis made from polyester fibers coated with a metal-polymer composition, including the stabilized SN in a concentration of 6.8 and 11.3 mg per 1 g of the hernioprosthesis mesh. During this research we used guinea pigs to study the *in vivo* tissue reactions. The clinical part of the study included the group of 212 patients who underwent removal of an inguinal hernia. We have identified various factors associated with infectious and toxic effects on the body by determining the level of the serum glutamate-pyruvate-transaminase (SGPT).

**Results:** In implantation of the hernioprotheses, including the high concentration of SN in the laparotomy wound, the exudative component of the inflammation was weakly expressed. It was mostly the proliferative changes that took place. We did not find either CD8-positive type T lymphocytes or PAX5-positive type B activated cells in the exudate.

**Conclusion:** Our research has shown that the use of hernioprotheses that include silver nanoclusters leads to the reduction of inflammation in the exudative phase and to a more favorable course of reparative processes.

**Keywords:** *stabilized silver nanoclusters; periprosthesis tissue; intraperitoneal location of hernioprosthesis; exudative component of the inflammation.*

### Introduction

There are studies in experimental surgery examining the tissue reactions to hernioprotheses made of various polymeric materials. Among them, special attention is paid to the work in which various substances, having not only antibacterial but also broader activity, are used as an additional pharmacological agent. These substances, particularly silver nanoclusters (SNs), interrupt the negative development of inflammation at the level of cytokines. In addition, the stabilized SNs give a better cosmetic effect in the treatment of skin burns. The acceleration of re-epithelialization and fibroblast differentiation has been noted during the experiments with those nanoclusters. The anti-inflammatory action was proven experimentally in a

serous membrane model with postoperative adhesions. This action was shown in mice having mechanical damage of the serous membrane [1-2].

**The aim** of our work was to elaborate the experimental model of prosthesis hernioplasty in subcutaneous and intraperitoneal locations of hernioprotheses with SNs, which allowed us to study the course of reparative reactions in all layers of the anterior abdominal wall. Additionally, we wanted to perform a comparative assessment of the use of polypropylene hernioprotheses and composite prostheses with SNs having antimicrobial effects.

### Material and Methods

We used a modified hernioprosthesis made from polyester fibers coated with a metal-polymer composition, including the stabilized SN in a concentration of 6.8 and 11.3 mg per 1 g of the hernioprosthesis mesh. Zero-valent metallic

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silver in combination with polyvinylpyrrolidone existed in a stabilized state, in the form of spherical nanoclusters with a limited particle size distribution in the range of 1–4 nm. The major part (over 80%) consisted of 1–2 nm silver particles. The adhesive composite was seated firmly on the fibers of the prosthesis, turning them into pseudomonothreads and eliminating their capillarity. After the implantation, the composite was dissolved gradually in the tissue fluid. Thus, the voids between fibers were liberated and that contributed to the effectiveness of connective tissue invasion.

A method for producing and manufacturing mesh prostheses for hernioplasty with antimicrobial properties (PSGA) completed with antibacterial suture material has been developed by the Plasmofilter company (St. Petersburg, Russia). It is intended for the use of plastics in abdominal hernia surgery (in cases of aponeurosis-muscle tissue defects). It can also be used for wound healing after the removal of a tumor and in chest or abdominal wall lesions, as well as other soft tissue defects.

During this research we used guinea pigs to study the *in vivo* tissue reactions. The hernioprosthesis was implanted intraabdominally into 5 animals with a concentration of 6.8 mg of SN per 1 g of the mesh. For 10 animals the concentration was 11.3 mg; the control group without a steeped hernia prosthesis contained 5 animals. The surgical interventions were carried out under conditions of contamination, without observing an aseptic operating regimen. The animals were removed from the experiment on the 14<sup>th</sup> day. We evaluated the clinical course and the autopsy data during the postoperative period. Standard methods were used for histological investigation: paraffin embedding and staining of the sections with hematoxylin and eosin, as well as Masson's trichrome method. We also used immunohistochemical markers for CD8, CD20, and PAX5. We evaluated the degree of sclerotic lesions, the severity and qualitative composition of the inflammatory infiltrate, as well as the repair tendency of the process. All procedures on animals were conducted according to European Convention ET/S 129 and directive 86/609 EEC.

The clinical part of the study included the group of 212 patients who underwent removal of an inguinal hernia. We have identified various factors associated with infectious and toxic effects on the body by determining the level of the serum glutamate-pyruvate-transaminase (SGPT). We carried out a comparative evaluation of SGPT complications. The study group consisted of 115 patients having the composition hernioprosthesis with SN. The control group consisted of 97 patients. Patients were informed about the details of the ongoing study and signed an agreement to participate in the research. We compared our results with the European Hernia Society experts' opinion [3]. These experts consider that up to now the ideal mesh prosthesis is unknown. We studied the frequency of various complications, such as seroma, blood leukocytosis, postoperative fever, festering wounds or inflammatory infiltrate, purulent fistula, foreign body feeling, use of antibiotic prophylaxis, and hernioprosthesis rejection.

Results were statistically processed using the software package Statistica. The mean (M) and standard deviation (SD) were deduced. Analysis of the distribution of values

obtained was performed using the Kolmogorov-Smirnov test. For data with normal distribution, inter-group comparisons were performed using Student's t-test. The Mann-Whitney (U Test) was used to compare the differences between the two independent groups (for nonparametric data). A value of  $P < 0.05$  was considered statistically significant.

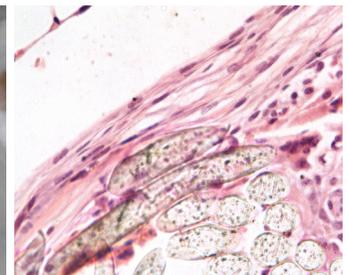
## Results

All animals that were implanted with the hernioprosthesis with SN had a favorable postoperating period. They behaved actively and had a good appetite. The skin wounds healed without inflammatory complications. No inflammatory changes in peritoneum were detected at the autopsy (Fig.1). While using the fragments of prostheses containing 6.8 silver mg per 1 mesh g, the point adhesion of the omentum majus to the prosthesis was marked. Neither point adhesion of the omentum to the wall nor other reactive changes in the abdominal cavity were detected during the autopsy of the animals, which had the hernioprosthesis containing 11.3 silver mg per 1 mesh g.

On the other hand, generalized and local reactions were noted in the complicated postoperating process in the control group. All 5 animals lost body weight and had limpness and a decrease in appetite. The toxicosis features were expressed clinically. The skin wounds were always healed by the secondary intention. At the autopsy in all cases the inflammatory infiltrate was detected intraabdominally in the area of the abdominal wall around the lavesan net including omentum, gut wall, and liver. During histological investigation of the tissues around both prosthesis types covered with SN, similar histological changes were defined. Foreign body granulomas consisted of a small amount of lymphocytes, and singular giant multinucleated cells were found around the prosthesis. The thin fibrous streaks were defined by the use of Masson trichrome staining; the new production of mesothelium and the formation of neoperitoneum were marked (Fig.2).



**Fig. 1.**  
Autopsy



**Fig. 2.**  
Mesothelium production  
around prosthesis

When using the hernioprosthesis without anti-inflammatory action, mainly the exudative inflammation period was detected in the laparotomy wound. The young granulation tissue and massive infiltration by polymorph-nucleated leucocytes were determined in the tissue that adhered to the net. Our immunohistochemical research confirmed the data of routine histology. In implantation of the hernioprosthesis, including the high concentration of SN in the laparotomy wound,

the exudative component of the inflammation was weakly expressed. It was mostly the proliferative changes that took place. We did not find either CD8-positive type T lymphocytes or PAX5-positive type B activated cells in the exudate (Fig.3). The number of the CD20-positive type B lymphocytes (Fig.4) was low and was reduced more with an increased concentration of the silver nanoclusters in the composition of the hernioprotheses. When the hernioprotheses were used without anti-inflammatory action, the exudative period of inflammation was detected primarily in the laparotomy wound; thereby, besides the large number of neutrophil granulocytes, the high concentration of CD8-positive type T lymphocytes was defined in the tissue.

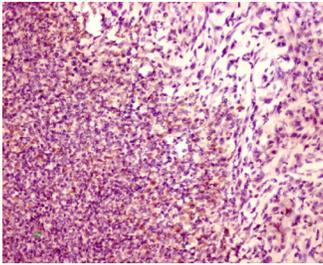


Fig.3.

*Polymorphonucleocytes and modest number of PAX5+ activated B-lymphocytes in the exudate.*

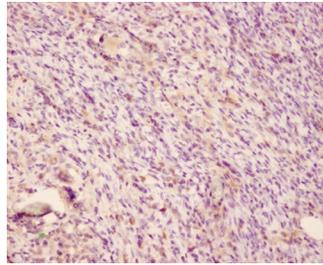


Fig.4.

*Polymorphonucleocytes and modest number of CD20+ B-lymphocytes in the exudate.*

There was no rejection of the hernioprotheses in either group during the period of experiment. For the control group patients, a standard antibiotic prophylaxis was performed, but no prophylaxis was done for the study group patients having the composition hernioprotheses with SN. Seromas were detected in 2 (1.7%) patients of the study group and in 8 (8.2%) patients of the control group,  $P < 0.05$ . We also noted leucocytosis in 7 (6.1%) patients of the study group and in 15 (15.5%) of the control group,  $P < 0.05$ . Postoperative fever was detected in 6 (5.2%) men of the study group and in 11 (11.3%) men of the control group,  $P < 0.05$ . Leukocyte infiltrate was detected in 1 (0.8%) patient of the study group and in 5 (5.1%) men of the control group,  $P < 0.05$ . Fistulas were noted in 2

(1.7%) patients of the study group and in 9 patients of the control group. The foreign body feeling was detected in 1 (0.8%) patient of the study group and in 12 (12.3%) men of the control group,  $P < 0.05$ .

## Conclusion

Our research has shown that the use of hernioprotheses that include silver nanoclusters leads to the reduction of inflammation in the exudative phase and to a more favorable course of reparative processes. These processes are characterized by the appearance of thin connective tissue streaks around the prosthesis and the new formation of mesothelium (neoperitoneum). Our immunohistochemical data allow us to suppose that the use of the hernioprotheses with anti-inflammatory action is able to activate certain components of humoral immunity. This supposition is confirmed by the increasing number of CD20- and PAX5-positive type B lymphocytes. These properties of hernioprotheses with SN cause a lack of fibrous adhesions (commissures) in the majority of experimental animals in which the prostheses were implanted. Complications resulting from use of the mentioned prostheses were detected more rarely than from use of polypropylene hernioprotheses. The use of stabilized nanoclusters seems to be a promising addition to the creation of composite polymer hernioprotheses.

## Competing interests

The authors declare that they have no competing interests.

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