Experimental Development of New Surgical Suturing Materials with Complex Biological Activities

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 153, No. 3, pp. 391-396, March, 2012
Original article submitted December 29, 2010

New surgical suturing materials with complex biological activities (antibacterial and stimulating tissue regeneration) have been developed. *In vitro* studies demonstrated pronounced and prolonged (up to 10-12 days) antibacterial activity. Experiments on 108 male albino rats proved the positive effect of the materials on the wound process: shortening of the inflammation period, more rapid transformation of the granulation tissue, more rapid epithelialization of the wound and its pronounced contraction.

Key Words: *suturing materials; antibacterial activity; regeneration*

Interventions in various spheres of surgery are often associated with local postoperative complications, particularly in grave weak patients, with characteristic reduction of tissue capacity to regeneration [8]. A promising trend in prevention of these complications is improvement of methods for tissue connection and the search for new suturing materials providing tissue union [2,7]. Modern chemical technologies allow creation of new bioactive suturing materials by incorporation of drugs into synthetic threads. These new materials prevent wound infection and improve tissue reparation [3,5,9,11,12]. Germanium-containing organic compounds (GOC) [1,6], for example, panaxel and astragerm, used in practical surgery [2], seem to be promising drugs improving tissue reparation. Therefore, the development of new more effective suturing materials characterized by complex effects and manufactured by simpler technologies is an important problem.

We summed up the experimental stage in development of surgical suturing materials with complex (antibacterial and regeneration-stimulating) activities.

MATERIALS AND METHODS

The study was carried out in collaboration with specialists from All-Russian Institute of Synthetic Fibers with an Experimental Plant (Tver). New nonabsorbable complex threads with coating impregnated with antibacterial drugs and GOC stimulating tissue reparation have been created [4]. A total of 180 specimens of the following groups of suturing materials were tested: 1) bioinert polycaproamide thread (PCAT) served as the control; 2) PCAT with antibiotic (doxycycline) and GOC (panaxel), PCATDGOC; 3) PCAT with antibacterial drug (ciprofloxacin) and GOC substance (astragerm), PCATCGOC.

The threads are manufactured by different technologies, which determine different degree and duration of their antibacterial activity. Therefore, we started from evaluation of their antibacterial effects *in vitro*. Antibacterial activity of suturing materials was evaluated directly after they were taken from package and after exposure in saline (which served as a model of living soft tissues) in a flow mode for 1, 3, 5, 7, 10, 12, and 15 days. Fragments of threads were removed from the model solution after these periods. *Staphylococcus aureus* 906, *Escherichia coli* K12, and *Bacillus subtilis* L2 strains (from collection of test cultures of Bacteriological Laboratory, Center of State Sanitary Epidemiological Surveillance in the Tver Region) were inoculated in solid nutrient media in Petri dishes. Specimens of the studied threads (2 cm long) were applied to the cultures and the preparations were incubated in a thermostat at 37°C for 24 h. Growth de-

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0007-4888/12/1533-0409

lay zones around the suture material specimens in bacterial cultures were then measured (in mm). Threads with the highest and longest antibacterial activities and made by the simplest technology were manufactured at the experimental plant for further *in vivo* studies (the suturing materials are manufactured in sterile polyethylene packages with shelf life of 5 years).

In vivo experiments were carried out on 108 male inbred albino rats. The cytology of the wound process under conditions of implantation of different suturing materials was studied in a special group of 42 animals. Experimental wounds (400 mm^2) on the dorsal surface were created similarly in all animals under ether narcosis. Specimens of suturing material (eight 2-cm fragments) were then implanted to a depth of 1-2 mm into the wound bottom tissues with a suture needle (innovation proposal No. 2386 of 28.06.2002). The wounds healed by second intention. Impression smears from the wound surface were collected by the method proposed by M. M. Pokrovskaya and M. S. Makarov [10] 12 h after wound infliction. After staining by Romanowskii's method, cytological study of the impressions was carried out, which showed the effects of implanted threads on the wound process inflammation phase (after M. I. Kuzin, 1977). Wound exudation cells were counted in impression smears under immersion microscope in 10 fields of view and their diameters were measured by an ocular micrometer.

The histomorphology of healing of linear wounds sutured by the test materials was studied in the rest 66 animals. Linear cut wounds (5 cm) of the skin with subcutaneous fat were inflicted in the same site of the dorsal surface of the trunk in animals under ether narcosis. The wounds were sutured by six individual nodular sutures. The material for analysis was collected by resecting the wound edge with the cicatrix on days 3, 5, and 7 after the operation. The sections (6-7 μ) were stained by hematoxylin and eosin. Histological preparations were examined under a light microscope at ×80.

The animals were divided into 3 groups; in each group, different suturing materials were used: control (PCAT), experimental 1 (PCATDGOC), and experimental 2 (PCATCGOC).

RESULTS

According to microbiological findings, antibacterial activity of suturing materials with doxycycline and panaxel (PCATDGOC) was retained during 10-12 days of exposure in model medium in the flow mode. After longer exposure in solution the threads lost their antibacterial activity because of complete removal of the drug from the specimen. These specimens were more active towards *St. aureus* 906 and *B. subtilis* L2 and less so towards *E. coli* K12 test cultures. Polycaproamide thread with ciprofloxacin and astragerm (PCATCGOC) exhibited pronounced prolonged (up to 15 days) antibacterial activity towards all experimental micro-organism strains. All these specimens exhibited the highest activity towards *E. coli* K12.

Cytological analysis of impression smears demonstrated significant differences in the qualitative and quantitative composition of wound microflora in animals of the three groups (Table 1). The exudation from controls (PCAT) contained numerous neutrophilic leukocytes, often with loose and enlarged nuclei. The neutrophils were evenly scattered in the smears. The mean diameter of a cell was 12.8±0.1 μ. Solitary (up to 2.9±0.1 per visual field) phagocytic macrophages, rather small (18.7±0.4 μ) with nuclei of irregular shape and round cytoplasms appeared (Fig. 1, *a*).

Analysis of the wound exudation from experimental groups 1 (PCATDGOC) and 2 (PCATCGOC) indicated more pronounced increase in the counts of neutrophils, which were much larger than in the control (the differences were significant; Table 1). The counts of actively phagocytizing neutrophils were negligible. The overwhelming majority of neutrophilic leukocytes were at different stages of physiological destruction. Clear-cut segmentation of their nuclei was violated. In some cells, the segments of the nucleus acquired a round shape and lost connection to each other, in others these segments fused into a homogenous formation.

TABLE 1. Counts (in 10 Visual Fields) and Diameters (in μ) of Wound Exudation Cells 12 h after Surgery (*M±m*)

Group	Neutrophils		Macrophages	
	count	diameter	count	diameter
PCAT	229.1±14.2	12.8±0.1	2.9±0.1	18.7±0.4
PCATDGOC	310.7±13.7*	16.5±0.3*	12.7±0.4*	25.5±0.2*
PCATCGOC	284.3±12.0*	14.2±0.3*	8.1±0.1*	23.4±0.4*

Note. *$p<0.05$ in comparison with the control (PCAT).

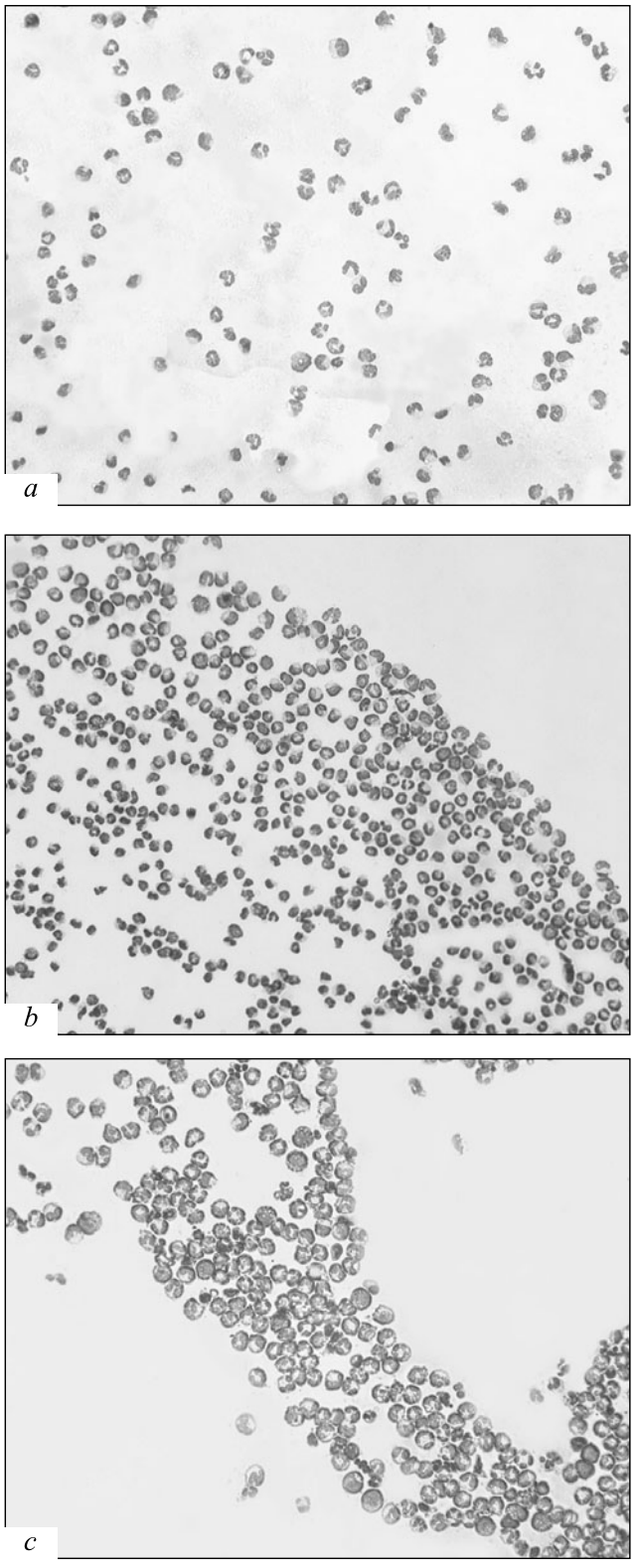

Fig. 1. Cytograms of impression smears from wounds with suturing materials implanted in the bottom. Romanowskii's staining, ×1000. *a*) control: neutrophils of normal structure and solitary macrophages in exudation 12 h postoperation; *b*) experimental group 1: macrophage accumulations with numerous digestive vacuoles and with neutrophils; *c*) experimental group 2: accumulations of phagocytic macrophages and degenerative changes in neutrophils.

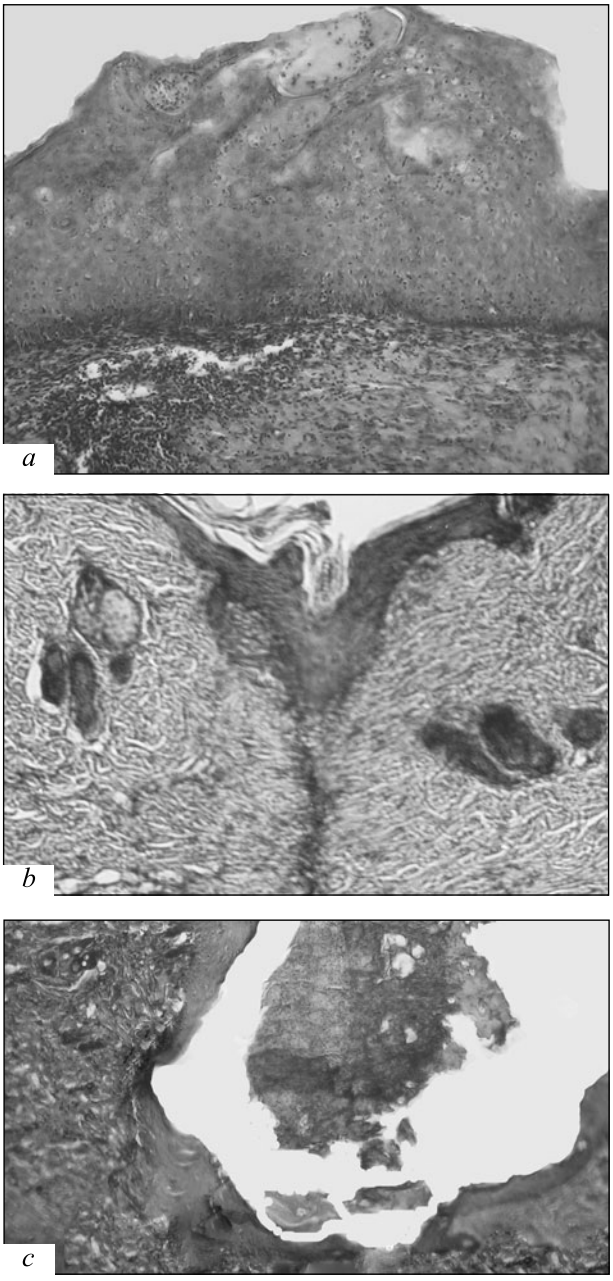

Fig. 2. Focus of injury 3 days after operation. Hematoxylin and eosin staining, ×80. *a*) control: leukocytic roll and forming granulation tissue; *b*) experimental group 1: formation of skin derivates in the wound; *c*) experimental group 2: new epithelium in focus of injury.

Fulminant reaction of macrophages was observed in animals of experimental groups: cell counts and size significantly increased. In experimental group 1, the macrophages counts in groups 1 and 2 were 12.7±0.4 and 8.1±0.1 per visual field, respectively, *vs.* 2.9±0.1 in the control. Macrophages were scattered evenly in a visual field or formed groups. The cells in experimental groups were significantly ($p<0.05$) larger than in the control. Numerous digestive vacuoles in the cytoplasm, looking like a honeycomb (Fig. 1, *b*,

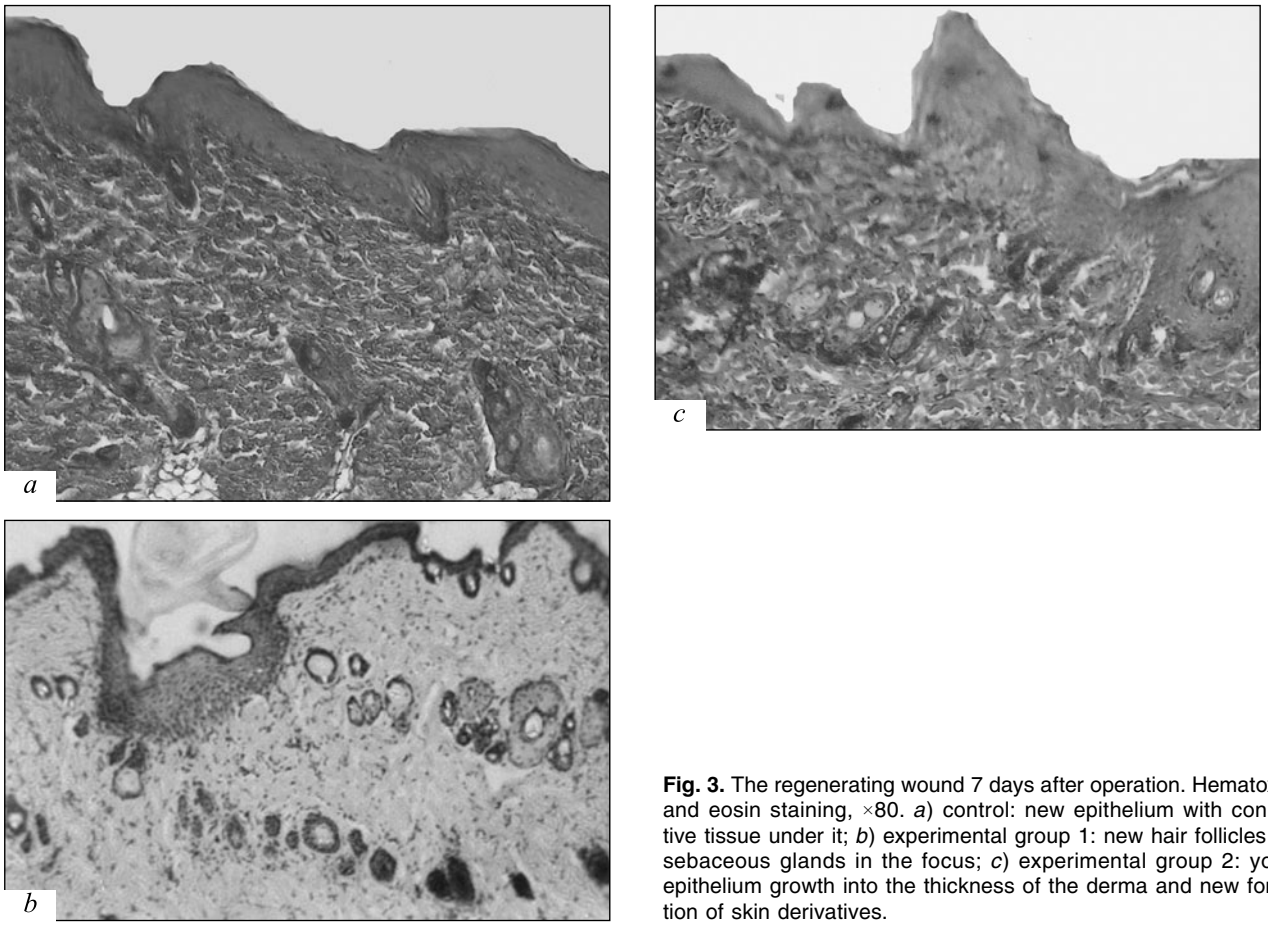

Fig. 3. The regenerating wound 7 days after operation. Hematoxylin and eosin staining, ×80. *a*) control: new epithelium with connective tissue under it; *b*) experimental group 1: new hair follicles and sebaceous glands in the focus; *c*) experimental group 2: young epithelium growth into the thickness of the derma and new formation of skin derivatives.

c) indicated high functional activity of macrophageal elements. Vacuoles sometimes contained rudiments of nondigested particles.

Hence, implantation of suturing material containing GOC (panaxel or astragerm) into the wound stimulated cell migration into the focus, paralleled by increase of their functional activity. This fact indicated significant stimulation of the inflammatory process in the presence of GOC, which nevertheless passed through all phases characteristic of it. Addition of GOC to suturing material resulted in an increase of the total count of neutrophilic leukocytes migrating to the focus and early emergence of macrophages.

Analysis of histological preparations also showed some differences between the groups. Three days after surgery, the wounds in the control group (PCAT) were covered with a compact, sometimes fragmented crust consisting of destroyed and degenerative cells (mainly neutrophilic leukocytes). A small leukocytic roll was found under the crust. The forming granulation tissue occupied the central area of the wound and consisted of numerous capillaries and round cells which formed focal accumulations (Fig. 2, *a*).

In experimental group 1 (PCATDGOC), the wound after 3 days was a small depression lined with new slightly hypertrophic epithelium. The epithelial layer

TABLE 2. Size and Numbers of Structures of Healing Wounds on Day 7 after Surgery (μ; *M*±*m*)

Group	New epithelium		Width of cicatrix	Number of skin derivatives in visual field
	thickness	length		
Control	138.1±5.2	443.5±25.6	39.9±1.6	9.3±1.3
Experiment	71.1±3.6*	317.8±24.1*	13.8±0.5*	15.1±0.9*

Note. *$p<0.05$ in comparison with the control.

consisted of 7-8 layers of cells with clear-cut structure. The basement membrane had protrusions with new hair follicles and sebaceous glands in some places (Fig. 2, *b*).

In experimental group 2 (PCATCGOC), a large fragmented crust partially covered the wound during the same period; sometimes the crust was completely detached. Mature granulation tissue containing various hematogenic and tissue cells was found in the center of the wound; solitary macrophages, histiocytes, fibroblasts, and collagen fibrils appeared by the wound edges, and the growth of young epithelium started, its basement membrane smooth or sometimes slightly bulging out into subjacent tissue. The new epithelium was hypertrophic in areas adjacent to intact tissue (Fig. 2, *c*).

All the differences noted previously manifested most clearly 5-7 days after surgery (wound process regeneration phase, after M. I. Kuzin, 1977). This was confirmed by morphometric values of the main structures in the postoperative wound (Table 2). Complete epithelialization was seen in control animals (PCAT). The new epithelial layer completely lined the defect area and consisted of several cell layers. The basement membrane was uneven, though did not bulging out into subjacent tissue. No skin derivatives were forming. Typical connective tissue was found under the epithelium (Fig. 3, *a*).

Organ-specific regenerated tissues with all structures characteristic of normal skin were found in animals of experimental group 1 (PCATDGOC). The epithelium was thin, consisted of 5-6 cell layers, and was plicated due to the wound contraction. The basal membrane looked uneven, had numerous protrusions into the subjacent tissue; active formation of elements was in progress in the protrusions (Fig. 3, *b*). New and forming hair follicles and sebaceous glands were seen in the healing areas of the wound. Their number increased significantly in comparison with the previous period. The postoperative cicatrix was fine and virtually unseen.

In experimental group 2 (PCATCGOC), skin wound healing eventuated in the formation of organ-specific regenerate: complete epithelialization, growth of young epithelium into the thickness of the derma, and new formation of hair follicles and sebaceous glands (Fig. 3, *c*). Folds on the surface of regenerated epithelium indicated wound contraction.

Hence, specimens of suturing materials with antibacterial drugs exhibited pronounced prolonged antibacterial effects. The antibacterial effect of PCATDGOC was more manifest towards *St. aureus* 906 and *B. subtilis* L2. PCATCGOC was active during a long period towards all the test micro-organisms. Addition of GOC to suture materials stimulated the course of wound process. Healing of linear wounds sutured by threads with GOC and antibacterial drugs was characterized by less pronounced and rapidly arrested inflammation, more rapid transformation of granulation tissue, wound contraction, and more rapid epithelialization of the defect.

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