Experimental Study of Reparative Regeneration Processes in the Wound Treated with Bioactive Dressings

I. A. Chekmareva

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 133, No. 2, pp. 226-230, February, 2002
Original article submitted October 30, 2001

Quantitative and structural functional analysis of granulation tissue cells during treatment with protein-polysaccharide dressing Collahit F was carried out. The preparation effectively cleansed the wound from detritus, prevented secondary infection due to stimulation of the functional activity of macrophages and due to the effect of its antiseptic component (furagin), and stimulated proliferative activity of fibroblasts and granulation tissue microvessels on day 5 of treatment, thus promoting repair processes in the wound.

Key Words: *bioactive wound dressing; protein-polysaccharide complex; granulation tissue; regeneration; electron microscopic autoradiography*

Creation of bioactive wound dressings (BAD), differentially modulating the course of the wound process, is a perspective trend in the development of new dressings. BAD are now used in the treatment of wounds at the regeneration stage with good results [1,6,7].

Collagen- and polysaccharide-based dressings are characterized by unique capacity to stimulate the reparative processes in the wound by creating conditions for fibroblast proliferation [1,6,7]. Collahit F is a collagen-chytosane complex containing 1% furagin.

Stimulating effect of protein-polysaccharide compounds on the functional activity of granulation tissue cells was described. We consider that the specific effect of a complex dressings on activity of GT cells is important for elucidation of the mechanisms of BAD effects on wound healing and for creation of new perspective BAD with target effects.

We investigated the specific effect of Collahit F, animal protein and polysaccharide-based BAD, on the ultrastructure and functional activity of GT cells.

MATERIALS AND METHODS

Experiments were carried out on 50 random-bred male albino rats. Wounds with aseptic inflammation were modelled under ether narcosis. Full-layer skin wounds (3 cm) were formed on animals' backs. After 2 days the crusts were removed from the wounds in experimental group animals and treatment with Collahit F BAD was started. In the controls the wounds healed under gauze dressings after removal of the crusts. GT specimens were examined on days 5, 7, and 9 after the wound modelling, which corresponded to days 3, 5, and 7 of BAD treatment. For autoradiography, GT fragments (1 mm^3) were incubated at 37°C in medium 199 containing 20 μCi/ml ^{3}H-thymidine (specific radioactivity 21.6 Ci/mM) or 100 μCi/ml ^{3}H-uridine (specific radioactivity 26 Ci/mM) for 1.5 h. Then the material was washed in cold medium 199, fixed in 2.5% glutaraldehyde and 1% OsO_4, dehydrated in ascending alcohols, and embedded in epon and araldite. Radioautographs of semithin sections and electron microscopic radioautographs were prepared [3,4] and examined under a Philips CM 10 electron microscope.

For comparative quantitative analysis, fibroblasts, macrophages, vessels, and numbers of ^{3}H-thymidine-labeled fibroblasts and vessels were counted under an optic microscope in 30 visual fields (100 mm^2 each) at ×1000. Mean values and percent ratios of proliferating to total number of cells and vessels were estimated. The results were statistically processed using Student's *t* test.

Department of Pathological Anatomy, A. V. Vishnevskii Institute of Surgery, Russian Academy of Medical Sciences, Moscow

0007-4888/02/1332-0192$27.00

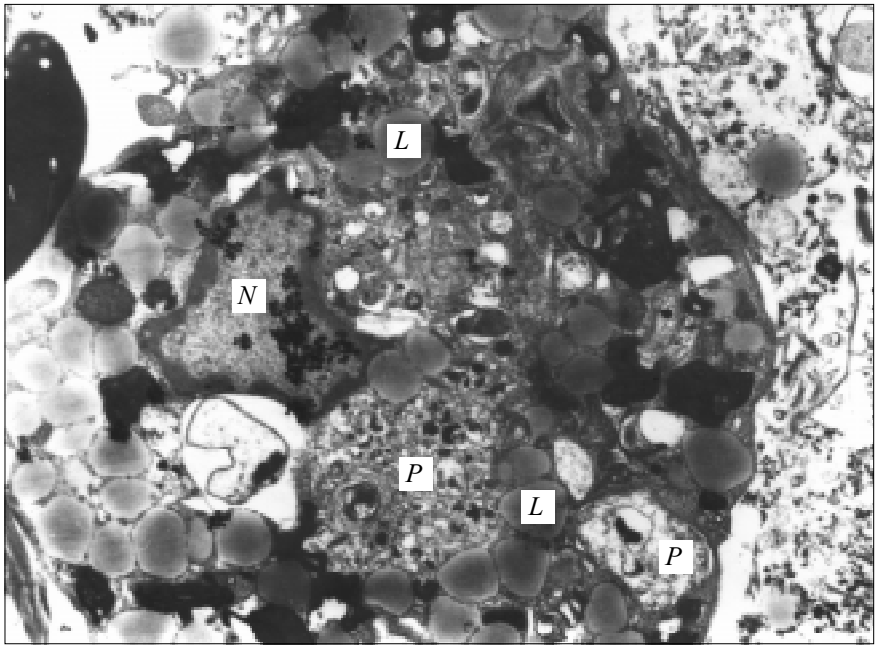

Fig. 1. Active phagocytosing macrophage in rat granulation tissue on day 3 of treatment with Collahit F dressing, ×9000. RNA synthesis (^{3}H-thymidine label) in the nucleus (*N*). Lipid droplets (*L*), phagosomes (*P*), residual bodies in cell cytoplasm.

RESULTS

On day 3, a thin layer of GT of scanty cellular composition formed only in the center of the wound in the subcutaneous fat. Poorly differentiated and proliferating fibroblasts and solitary vessels were seen among adipocytes. In the experimental group the majority of GT macrophages were in an active state on day 3 of treatment, which was confirmed by the presence of ^{3}H-uridine label above the nucleus (RNA synthesis) and characteristic ultrastructure of the cells (Fig. 1). Macrophage cytoplasm contained numerous phagosomes with cell detritus and myelin-like figures. This structure indicated high phagocytic activity of macrophages. In the controls GT was abundantly infiltrated by polymorphonuclear leukocytes with numerous phagosomes with bacterial and cell detritus. Active macrophages were less numerous than in experimental animals.

In experimental animals the number of macrophages was maximum on day 3 of treatment and then decreased (Table 1). In controls the number of macrophages in GT increased until complete healing. Decreased number of macrophages in the wounds of experimental animals can be explained by their higher functional activity, which determined more effective wounds cleansing and reduced inflammation.

No activation of proliferative processes in GT was observed on day 3 of treatment with Collahit F BAD.

On day 5 of treatment the number of proliferating fibroblasts sharply increased in the experi-

TABLE 1. Time Course of Cell Elements and Vessels in Granulation Tissue Experimental Wound during Treatment with BAD (*M*±*m*)

Cell type and vessels	Day of treatment					
	3		5		7	
	experiment	control	experiment	control	experiment	control
Fibroblasts						
total	9.60±0.68	8.47±0.79	20.03±1.31*	9.90±0.77	12.90±0.62	15.03±0.91
of these, proliferating	0.70±0.22	1.27±0.26	4.40±0.66*	1.40±0.28	0.83±0.17	0.93±0.18
Vessels						
total	1.07±0.07*	1.73±0.25	1.53±0.13	1.87±0.19	3.33±0.33*	1.53±0.19
of these, proliferating	0.70±0.07	0.60±0.16	0.80±0.14*	0.33±0.16	0.73±0.21	0.53±0.22
Macrophages	2.63±0.36	2.60±0.22	2.32±0.20	3.43±0.27	1.77±0.29	4.03±0.38

Note. *$p<0.05$ *vs.* the control.

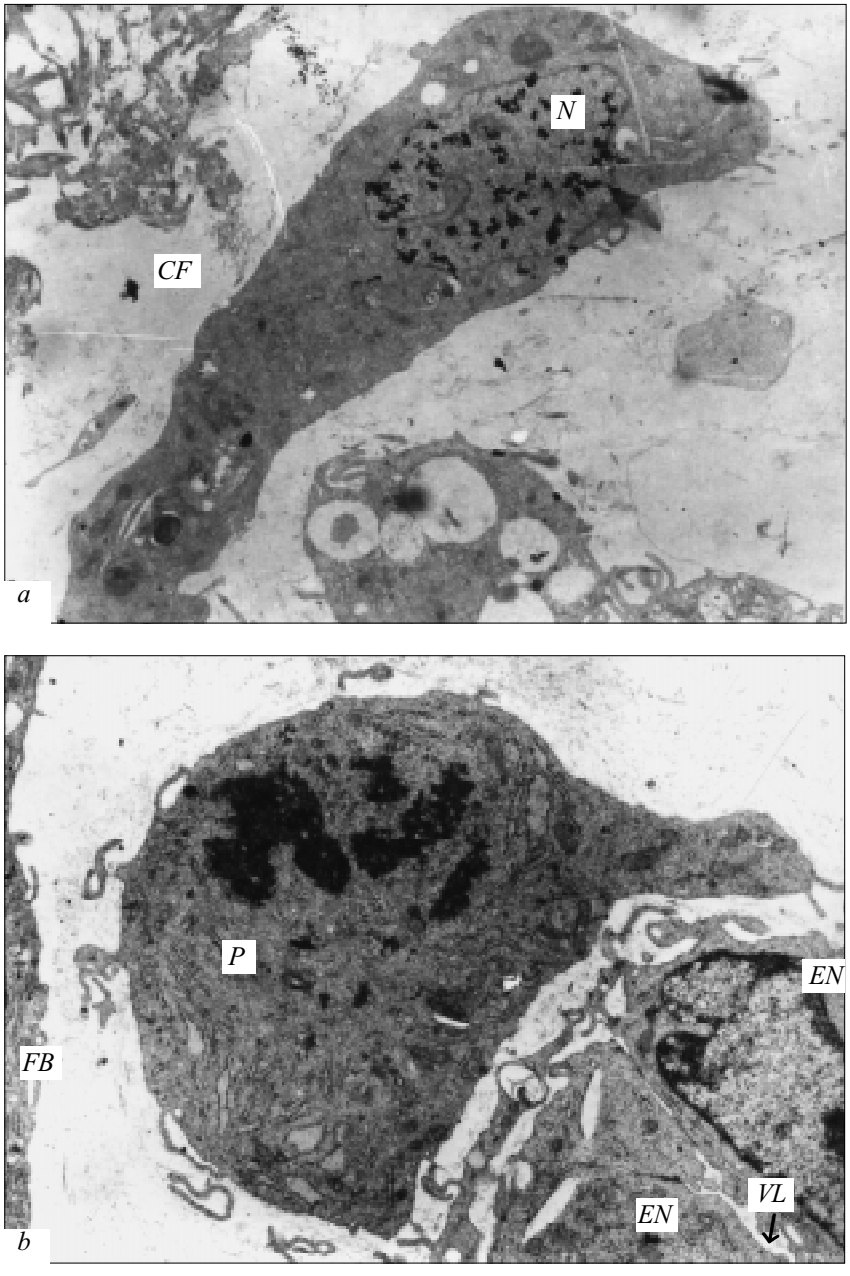

Fig. 2. Fragment of granulation tissue on day 5 of treatment with Collahit F dressing. *a*) proliferating fibroblast. ^{3}H-thymidine label (black silver grains) above the nucleus (*N*) indicate intense DNA synthesis; *CF*: collagen fibers, ×8500; *b*) capillary pericyte (*P*) in the stage of mitosis. *FB*: fibroblast; *VL*: vascular lumen; *EN*: endotheliocyte, ×9000.

mental group in comparison with the previous term (6.3 times, $p<0.05$) and with the control (3.14 times, $p<0.05$). Proliferating fibroblasts had a characteristic structure (elongated, with narrow cisterns of the granular cytoplasmic reticulum, well developed Golgi complex, large nucleus with ^{3}H-thymidine label) and were often surrounded by synthesized fine collagen fibers (Fig. 2, *a*). As a result of increased number of proliferating fibroblasts, the total number of fibroblasts (proliferating and not) in GT increase, and peaked on day 5 of treatment (Table 1).

During healing GT consumes more nutrients than normal connective tissue. Local circulation in the wound is insufficient, and the only way to deliver nutrients to tissues is the formation of new blood vessels.

In the experimental group the maximum stimulation of proliferative activity of vascular cells was observed on day 5 of treatment, while on day 7 the total number of vessels was maximum. In the control the number of growing vessels increased on day 9 (Table 1).

On day 5 the increase in proliferative activity in experimental group was paralleled by an increase in functional activity of endotheliocytes and pericytes, which was seen from RNA synthesis in their nuclei and ultrastructural changes in endothelio-

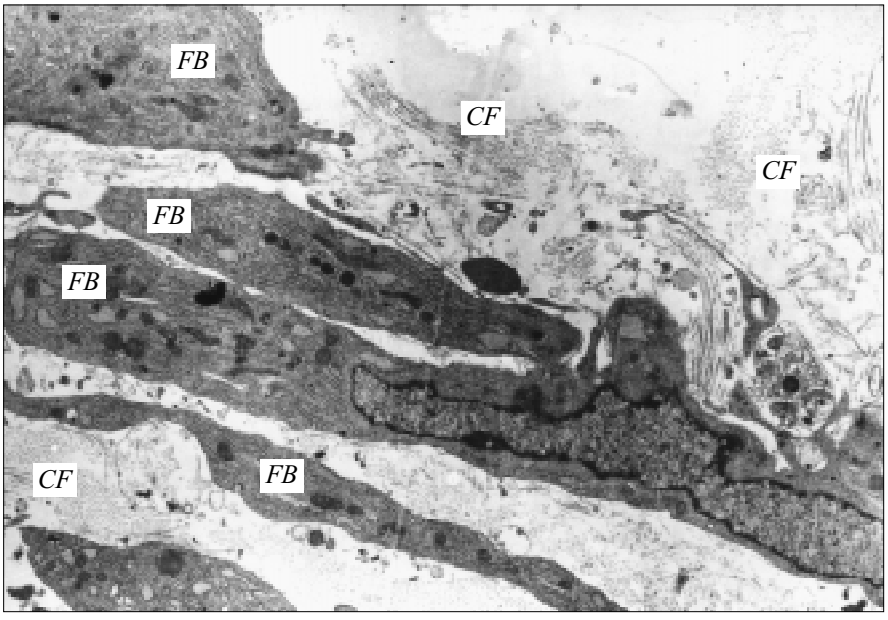

Fig. 3. Mature granulation tissue on day 7 of wound treatment with Collahit F dressing, ×4300. *CF*: collagen fibers; *FB*: fibroblast.

cytes: formation of numerous vesicular structures in the luminal surface, indicating intensification of transport processes in vascular wall cells and hence, improvement of GT trophics.

In addition, we observed mitosis in the microvessel pericyte (Fig. 2, *b*), which proves the involvement of pericytes in the formation of new cell elements in GT. A. V. Smol'yannikov *et al.* [5] consider that cells appearing after pericyte division leave the vascular wall and can differentiate into fibroblasts.

The oscillations of the curve reflecting the number of proliferating fibroblasts during wound treatment with Collahit F BAD were synchronous with fluctuations in the number of capillaries. On day 5 of treatment the peaks of proliferative activity of vessels and fibroblasts coincided. This synchronization can be regarded as a result of general stimulating effect of BAD and a result of histogenetic relationship between GT fibroblasts and small vessels [4]. Hence, capillaries play an important role in supply of nutrients to growing GT and can be the sources of cells for newly forming connective tissue.

On day 7 activity of mature GT in experimental group increased, while the proliferative activity of fibroblasts decreased 5.5 times in comparison with the previous term ($p<0.05$). Collagenoblasts surrounded by collagen fibers of different degree of maturity predominated in GT (Fig. 3). The degree of GT maturity increased from the wound surface to the bottom and from the center to the periphery. Marginal regeneration of the epithelium was observed at a small area.

In the control GT was notably infiltrated by active macrophages, whose cytoplasm was vacuolated and looked "spongy". No epithelialization foci were seen.

The results of our quantitative and structural and functional analysis of GT cells suggest that protein-polysaccharide dressing Collahit F effectively cleansed the wounds from detritus, prevented secondary infection due to stimulation of macrophage functional activity and its antiseptic component furagin, and stimulated proliferative and functional activities of GT fibroblasts and capillaries on day 5 of treatment, which accelerated repair processes in the wound.

REFERENCES

1. R. G. Muradyan, I. A. Chekmareva, A. A. Adamyan, *et al.*, *Byull. Eksp. Biol. Med.*, **120**, No. 11, 529-531 (1995).
2. D. S. Sarkisov, A. A. Pal'tsyn, and B. V. Vtyurin, *Adaptive Restructuring of Biorhythms* [in Russian], Moscow (1975).
3. D. S. Sarkisov, A. A. Pal'tsyn, and B. V. Vtyurin, *Electron Microscopic Radioautography of the Cell* [in Russian], Moscow (1980).
4. D. S. Sarkisov, E. G. Kolokol'chikova, and A. A. Pal'tsyn, *Byull. Eksp. Biol. Med.*, **103**, No. 6, 730-732 (1987).
5. A. V. Smol'yannikov, D. S. Sarkisov, and A. A. Pal'tsyn, *Arkh. Patol.*, No. 1, 3-13 (1984).
6. J. Noordenbos, C. Dore, and J. Hansbrough, *J. Burn Care Rehabil.*, **20**, Pt. 2, 230 (1999).
7. C. C. Van Gils, B. Roeder, S. M. Chesler, and S. Mason, *J. Am. Pediatr. Med. Assoc.*, **88**, No. 9, 452-456 (1998).