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AUTORADIOGRAPHIC AND ELECTRON-MICROSCOPIC INVESTIGATION OF THE EFFECT OF SOME SOVIET DRESSING MATERIALS ON EXPERIMENTAL WOUND HEALING

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Interest in the study of different aspects of wound healing has increased appreciably in recent years. To some extent this is due to the fact that many new techniques are now available for the research worker [3, 5-7]. However, in a field such as the development of new dressing materials, modern methods of morphological control have so far hardly been used at all. It is evident that the use of electron microscopy and autoradiography would help both to reveal structural differences in the course of wound healing taking place under different dressings, and also to verify the functional properties of dressings.

In the investigation described below some Soviet dressing materials were evaluated by autoradiography and scanning electron microscopy during use in experiments on animals.

EXPERIMENTAL METHOD

Experiments were carried out on 20 noninbred albino rats weighing 150-170 g, on which full-thickness excised wounds measuring 1.5 × 1.5 cm were inflicted in the dorsal region under ether anesthesia. The animals were divided into four groups. In group 1 (control) traditional cotton and gauze dressings were used, in group 2 the wounds were dressed with a double layer of atraumatic nontissue material, in group 3 cotton and gauze dressings also were used but Kapron gauze modified with a surfactant was chosen, and in group 4 dressings made from Kombutek-II were used. The dressings were changed every 2 days. During the dressing procedure the area of the wounds was determined by planimetry and squash preparations were taken from their surfaces. Biopsy material taken from the wounds was investigated once only, on the 8th day after the operation. To obtain autoradiographs, 5-[^{3}H]uridine (specific activity 18 Ci/mmole) was used as RNA precursor; fragments of tissue measuring 2 × 2 × 2 mm were incubated in medium with the precursor for 3.5 h at 37°C. After incubation the material was fixed with 15% formalin solution. Autoradiographs were obtained on paraffin sections 2-5 μ thick by the ordinary method using type M photographic emulsion. The number of tracks above the nuclei and cytoplasm of 100 fibroblasts was counted in sections stained with hematoxylin and eosin. The numerical results were subjected to statistical analysis by Wilcoxon's test.

Samples of dressing materials before and after application to the wounds were investigated with the scanning electron microscopy. The dressings were fixed with 3% glutaraldehyde solution in phosphate buffer, dehydrated in alcohols of increasing strength, and dried. The contact surfaces of the materials were sprayed with silver and examined in the ISM-2 scanning electron microscope.

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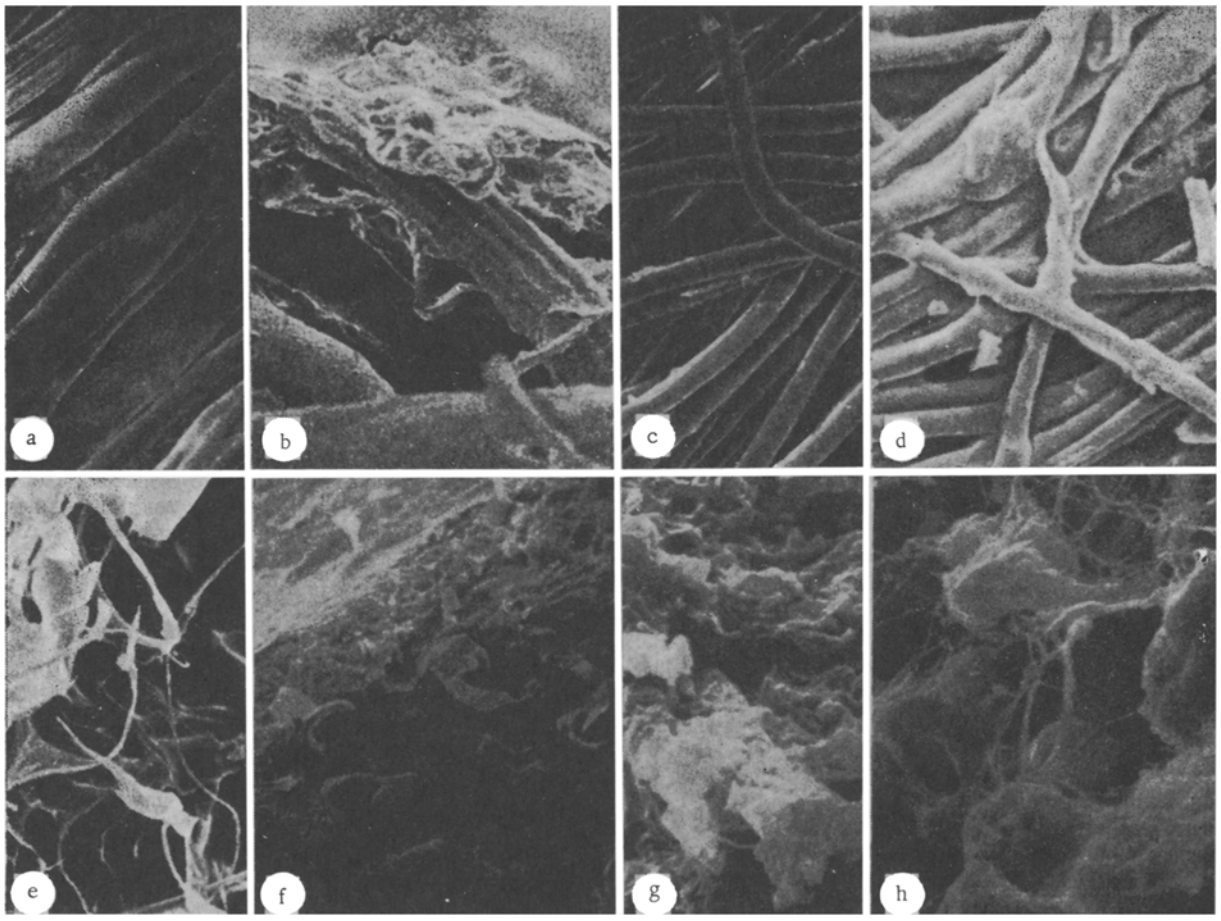

Fig. 1. Scanning electron microscopy of dressing materials before and after application to the wounds: a) cotton and gauze dressing (control) before application to wound; b) the same after removal from wound surface, 1000 ×; c) Kapron gauze modified with surfactant, before application to wound; d) the same after removal from wound surface, 300 ×; e) Kombutek-II dressing before application to wound; f) the same after removal from wound surface, 300 ×; g) fibrin clots in thickened layer of Kombutek-II, 3000 ×; h) area of wound surface on 8th day after operation (under Kombutek-II), 3000 ×.

EXPERIMENTAL RESULTS

The experiments showed that by the 8th day after the operation granulation tissue was sufficiently well formed in the wounds of animals of all the groups and filled the wound defect. At the edges of the wounds active epithelization was observed. In the first three groups of rats no significant histological differences could be detected. The nonepithelized areas of the wounds were covered with a leukocytic-necrotic barrier of roughly uniform thickness. Beneath it lay granulation tissue without any signs of inflammation. The regenerating epidermis crept over it in a rather thick layer. The thickness of this layer decreased away from the center of the wounds toward their edges because of the regular disposition of the cells of the newly formed epidermis and its differentiation.

Cells of the granulation tissue consisted mainly of young forms of fibroblasts, polyblasts, and undifferentiated cells of the perivascular cambium. The presence of a moderate number of leukocytes and macrophages was observed only in the upper layers of the wounds. Granulation tissue in the wounds of the rats of group 4 was distinguished by its greater compactness. Mature fibroblasts predominated in it, there were fewer polyblasts, but the density of distribution of the cells was higher than in the animals of the other groups.

The results of planimetry showed a more rapid decrease in the areas of the wounds healing beneath the test dressings compared with the control. The area of the wounds in all the experimental animals was 20-25% less than in the control.

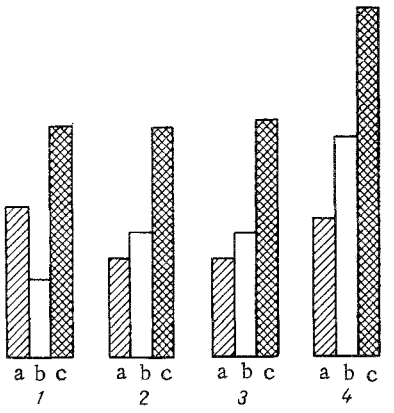

Fig. 2. Intensity of incorporation of 5-[^{3}H]uridine into fibroblasts of granulation tissue of wounds in animals of different groups: a) number of tracks above nuclei of 100 fibroblasts; b) number of tracks above cytoplasm of 100 fibroblasts; c) total number of tracks above 100 fibroblasts. 1-4) Groups of animals.

Histological examination of dressings removed from the wounds showed the presence of a varied number of areas of leukocytic-necrotic barrier and deposits of fibrin on their surface.

The results of investigation of the traditional cotton and gauze dressing and the double-layered atraumatic nontissue material by the scanning electron microscope were approximately the same. Fibrin clots and wound exudate covered the meshes and pores in the material and were closely apposed to the hydrophilic fibers. Particularly dense deposits were observed in layers adjacent to the wound (Fig. 1a, b).

Very small areas with deposition of fibrin were observed on the contact surface of gauze modified with surfactant. Most of the surface of the material remained clean (Fig. 1c, d). A dressing of Kombutek-II, when applied to wounds, underwent swelling and condensation in areas in contact with the wound surface. The thickness of the contact layer increased to 25-50 μ. Clots of wound secretion were present in the substance of the dressing (Fig. 1e-h).

It can accordingly be concluded from these results that all types of new dressing materials tested have a beneficial effect on regenerative processes in experimental wounds, although these properties differed. For instance, Kapron gauze modified with surfactant possessed good atraumatic properties, but the absorbent properties of the double-layered nontissue atraumatic material and of dressings made from Kombutek-II was quite considerable. The latter, in addition, had a wound-healing action which depended both on the presence of collagen breakdown products and of added medicaments [4]. The mechanisms of interaction between these dressings and wound surfaces evidently differed in each case. Cytological investigation of wound squash preparations revealed a marked macrophagal reaction on the wound surfaces of the rats of group 4 on the 5th day after the operation. Meanwhile a fibroblastic reaction was actively in progress on the wound surfaces of the animals of group 2, whereas in the rats of group 3 on the 5th day of wound healing only solitary fibroblasts were present on their surface and macrophages were infrequently found.

Analysis of incorporation of 5-[^{3}H]uridine showed that the total number of tracks above fibroblasts in wounds on animals of the first three groups was almost the same. In group 4 the number of tracks was almost 50% greater, indicating the more rapid synthesis of RNA by fibroblasts of wounds healing beneath a Kombutek-II dressing. When tracks were counted separately above nuclei and cytoplasm of these cells, the number of tracks above nuclei in the control group was found to be significantly greater than their number above the cytoplasm. In wounds of rats of the other three groups the number of tracks above the cytoplasm was appreciably increased and the number above the nuclei of the fibroblasts was reduced. This was seen particularly clearly in the animals of group 4 (Fig. 2). As previous investigations showed, a similar increase in the rate of nucleo-cytoplasmic transport of newly formed RNA took place in fibroblasts in wounds of animals receiving various stimulators of regeneration [1, 2]. A similar modification to the rhythm of biosynthesis and its activation may perhaps also take place in the fibroblasts of wounds healing under dressings promoting the more rapid completion of wound healing than in the control.

The results of this investigation thus suggest that the preparation of dressings from the samples of new Soviet materials tested is a promising development. It must be pointed out, in particular, that the most promising course would be to produce dressings with an action promoting wound healing, like Kombutek-II. The use of a combination of methods of morphological investigation is an essential step in the evaluation of the functional properties of new dressing materials.

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