TESTING PROMISING DRESSING MATERIALS ON CULTURES OF HUMAN SKIN FIBROBLASTS AND KERATINOCYTES

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With the appearance of a new generation of dressing materials, with assigned biologically active properties, greater demands are being placed on methods of their evaluation, including from the standpoint of their effect on proliferation and regeneration. The effective development of methods of culturing human epidermal keratinocytes [7] has made it possible to use human epidermis, reconstructed in vitro, in order to study various aspects of the toxicity of dressing materials, external environmental factors, drugs, and perfumery goods [2, 4, 5, 8, 9]. With the aid of cell cultures it is possible to conduct investigations on normal human keratinocytes, using a large quantity of homogeneous materials, which in some cases may have advantages over studies on laboratory animals.

In the investigation described below cultures of human skin keratinocytes and fibroblasts were used to test dressing materials in the form of sponges, made up on the basis of collagen. The criterion of suitability of these materials for use in medical practice was incorporation of labeled thymidine into the cultured cells, evidence of their proliferative activity.

EXPERIMENTAL METHOD

Spongy coverings based on a protein–glyc[illegible]aminoglycan complex (IK-4) and containing glycoproteins additionally (IK-7) were used for the investigation. The sponges wer[illegible] 0.5 mm thick, and their porosity and biodegradation in laboratory animals were monitored for 23 ± 1 days. Small disks were excised from the sponges aseptically and placed in flasks with cells. Intact sponges were used, but in one case they were washed three times with sterile Hanks' solution containing 1000 U/ml of penicillin and 0.5 mg/ml of streptomycin. Experiments were carried out on embryonic and postnatal human skin fibroblasts, generously provided [illegible] Freidin (Research and Production Center for Medical Genetics, Academy of Medical Sciences of the USSR). Fibroblasts (from 5 to 15 passages) were cultured in Eagle's medium with 5% fetal calf serum and 5% human umbilical serum at 37°C in an atmosphere of 5% CO_2 in air and of saturating humidity. Keratinocytes were isolated from adult donors and cultured as in [6] with minor modifications. Biopsy specimens of skin were generously provided by N. S. Rudenko (Research and Production Center for Cosmetics, Ministry of Health of the RSFSR). The action of the sponges on skin cell proliferation in culture was assessed by the addition of ^{3}H-thymidine (0.5 μCi/ml, specific activity 48.6 Ci/mmole). After the end of incubation with labeled thymidine the cells were washed twice with Hanks' solution, and then twice in the course of 10 min they were treated with a cold solution of 5% trichloroacetic acid. The cells were lysed in 0.1 N NaOH and added to scintillation fluid. Radioactivity of the incorporated ^{3}H-thymidine was determined on a "Tracor Analytic" counter (USA). The following cell systems were used to test the sponges. Fibroblasts were grown

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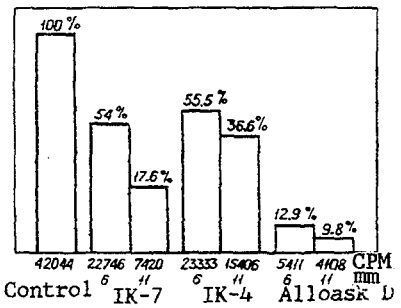

Fig. 1

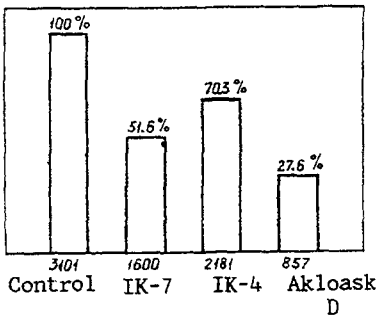

Fig. 2

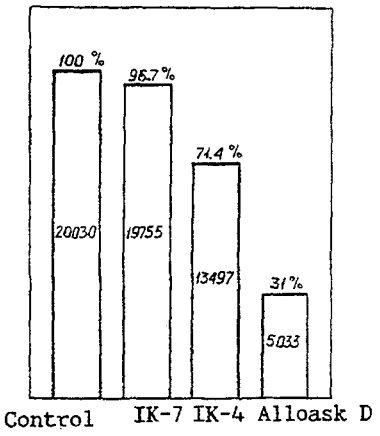

Fig. 3

Fig. 1. Effect of IK-4 and IK-7 Sponges on DNA synthesis in embryonic human skin fibroblasts. Fibroblast proliferation was stimulated by changing the nutrient medium containing ^{3}H-thymidine (0.5 μCi/ml), and IK-4 and IK-7 sponges 6 and 11 mm in diameter were introduced simultaneously.

Fig. 2. Effect of IK-4 and IK-7 Sponges on DNA synthesis after infliction of "sound" on monolayer of human embryonic skin fibroblasts. After "wounding," the cultures were washed and specimens of sponges introduced together with fresh nutrient medium, with the addition of ^{3}H-thymidine (0-5 μCi/ml).

Fig. 3. Effect of IK-4 and IK-7 sponges on DNA synthesis in basal keratinocytes of human skin. Proliferation of layer of basal cells was stimulated by a change of medium with simultaneous introduction of specimens of sponges and ^{3}H-thymidine (0.5 μCi/ml).

in plastic planchets, and after 48 h specimens of sponges were introduced together with fresh medium into the wells. Incubation with labeled thymidine continued for 24 h. In other experiments cultures of fibroblasts were preincubated in medium without serum, after which proliferation was stimulated by transferring the culture into fresh nutrient medium with serum. Meanwhile samples of sponges were added to the cultures with labeled thymidine for 24 h. Tests also were carried out on confluent cultures of fibroblasts, in which 25% of the cell layer was removed mechanically with a Teflon spatula, before the nutrient medium was changed and samples of sponges and labeled thymidine added. Finally, the effect of the test materials on keratinocyte proliferation was studied on a model of regeneration of the epidermis in vitro [3]. The method was as follows. After growth of a confluent layer of keratinocytes in medium MEM:F12 (1:1) with 10% fetal calf serum and with the addition of 10 ng/ml of epidermal growth factor, 6 μg/ml of insulin, and 10^6 M isoproterenol ("Sigma") the cultures were incubated for 3 days in medium 199 without Ca^{2+} and serum, as a result of which the suprabasal cells were detached and could be removed, Next, medium MEM:F12 was added again to the cultures with the addition of cell growth-stimulating factor, which led to the initiation of prolifer-

ative processes in the basal keratinocytes, followed by stratification of the culture. Together with fresh medium, samples of sponges were added to the flasks and, after 24 h, labeled thymidine was added for 24 h.

EXPERIMENTAL RESULTS

The results of experiments to study the effect of IK-4 and IK-7 sponges on proliferation of human skin fibroblasts are given in Fig. 1. It can be concluded from these results that unwashed sponges considerably inhibit incorporation of labeled thymidine into fibroblasts compared with control cultures (Fig. 1), whereas washed sponges had virtually no effect on incorporation of labeled thymidine. An increase in area of the discs excised from the sponges caused further inhibition of thymidine incorporation, evidence of suppression of proliferation. Incidentally, both specimens of sponges were less toxic for fibroblasts than the Alloask D, which is widely used as a wound covering. Suppression of fibroblast proliferation is evidently connected with the presence of toxic substances in the sponges, which can be removed virtually completely by washing.

Experiments were next carried out to study the action of the sponges on DNA synthesis in the damaged fibroblast monolayer. This model differs from that used previously in that in this case DNA synthesis takes place mainly in cells migrating from the "wound" edges to the free surface of the substrate [1]. The results (Fig. 2) show that IK-4 sponge inhibits DNA synthesis in human fibroblasts by a rather lesser degree than the IK-7 sponge; both sponges, moreover, were found to be less toxic than Alloask D.

Both sponges were found to inhibit DNA synthesis only very slightly in basal keratinocytes stimulated for proliferation, whereas Alloask D was much more toxic.

The results demonstrate that the dressing materials which were studied possess on the whole low toxicity for skin cells; the IK-7 sponge, moreover, inhibits keratinocyte proliferation to some degree, whereas the IK-4 sponge, conversely, blocks proliferation of fibroblasts more intensively. The results of these experiments confirm the real possibility of differential evaluation of the quality of dressings on the basis of their action on different types of skin cells.

By using human cell cultures it is also possible to study the action of dressing materials on protein synthesis, on contraction of collagen gel by fibroblasts, and on migration of cells and healing of wounds inflicted on a living skin equivalent, thus greatly increasing the practical value of the method.

The investigations showed that cultures of human skin keratinocytes and fibroblasts can be successfully used for testing dressing materials and for the treatment of wounds, especially burns. The cell cultures used, which constitute a highly sensitive testing instrument, enables strict selection of dressing materials to be undertaken, including those suitable for use during autografting of layers of keratinocytes grown in culture on to the wound surface.

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