# Reepithelization of Skin Wound under Collagen-Derived Coating

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The process of wound healing is studied after transplantation of skin micrografts covered by various collagen membranes. Cytotoxicity of membranes, the rate of reepithelization, and histological and histomorphometrical characteristics of the neoskin are analyzed.

**Key Words:** *reepithelization; skin microautograft; neoskin*

Dermoplasty in extensive skin burns and wounds is difficult because of donor's skin deficiency. In these cases healing can be achieved by using biotechnological approaches such as keratinocyte culture [5, 7,9] and skin organ culture [3,4,10]. The available biomaterials (donor or cadaver skin and porcine skin) are rejected, so they can be used only temporary [1,2]. Moreover, their production requires the development of special skin banks and analyses to exclude viral contamination, which raises their costs [6,8]. The present study is devoted to the search for optimal physiologically active nonimmunogenic membranes promoting wound healing. To this end we tested some commercial collagen membranes.

## MATERIALS AND METHODS

The following preparations were tested: kombutek-2 (Protein Sausage Casing Plant, Luzhsk), hemostatic collagen sponge (HCS, Belkozin Plant, Luzhsk), and Alloask D (porcine skin, Japan). Toxicity of these membranes was tested of the following cell cultures: embryonic human fibroblasts (EHF), human epithelioid kidney cells (Rh-Pi), and embryonic mouse fibroblasts (3T3). Cytotoxicity was evaluated by cell viability (Trypan Blue exclusion test), morphological parameters, and mitotic activity (hematoxylin and eosin staining).

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Collagen membranes were tested in a model of dorsal tangential skin lesion (under hexenal narcosis). Reepithelization was induced by transplantation of skin microautografts onto injured area, after which the wound was covered by one of the tested collagen membranes.

Three weeks postoperation the animals were sacrificed, and injured area together with collagen membrane was incised and subjected to histological examination. Possible effect of collagen on regeneration and reepithelization was evaluated by histomorphometrical method using a grid-ocular with 289 intersections and a 0.5 mm distance between them. Thickness of neoepidermis was measured with an ocular-micrometer.

## RESULTS

HCS proved to be the most toxic membrane. Morphofunctional examination of cell cultures showed that different collagen substrates had different effects on cell growth. For instance, a dense monolayer developed in the presence of porcine skin and kombutek, but not in the presence of HCS. None of these substrates induced cell destruction (Table 1).

Visual examination 10 days postoperation showed crusting and necrosis of alloskin. This period was long enough to trigger reepithelization, which was complete 2-3 weeks postoperation. By contrast, porcine skin remained practically unchanged throughout the entire experiment. In wounds covered with kom-

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TABLE 1. Mitotic Activity (‰) of Cells Cultures in the Ppresence of Collagen Samples

| Biomaterials | Cells | | |
|---|---|---|---|
| | HEF | Rh-Pi | 3T3 |
| Alloskin | 2.1 | 10.1 | 6.8 |
| Kombutek | 1.5 | 2.5 | 6.8 |
| Alloask D | — | 5.3 | 6.3 |
| HCS | 0 | 4.2 | — |

Note. HEF: human embryonic fibroblasts, Rh-Pi: epithelioid human kidney cells, 3T3: mouse embryonic fibroblasts.

TABLE 2. Effect of Different Collagen Membranes on the Parameters of Neoskin (*M±m*)

| Biomaterial | Thickness of epidermis, mm (without horny layer) | Weight, % | |
|---|---|---|---|
| | | intercellular substance | vessels |
| Intact skin | 0.02±0.002 | 78.0±1.6 | 3.0±0.4 |
| Alloskin | 0.1±0.004* | 59±11.0 | 7.8±0.5* |
| Kombutek | 0.05±0.009* | 63.3±13.8* | 3.5±0.5 |
| Alloask D | 0.04±0.005* | 59.8±2.0* | 3.4±0.3 |
| HCS | 0.05±0.006* | 62.8±1.5 | 2.8±1.0 |

Note. *$p<0.05$ compared with the control.

butek or HCS we observed shrinking and induration, and bleeding of wound edges during the first 10 days, as well as the absence of reepithelization of the open wound surface.

Alloskin and porcine skin ensure 100% reepithelization. There were solitary leukocytes, macrophages and mast cells in the derm. Granulation tissue consisted of young connective tissue judging from the presence of young fibroblasts and abundant microvascular system. There were no skin appendages, and hyperplastic neoepidermis contained all layers. A leukocyte-containing layer occurred between neoepidermis and a collagen membrane. On histological sections kombutek and HCS looked like dense fibrous structures sodden with extracellular fluid and erythrocytes and outlined with a leukocytic border. All parameters of neoskin were similar to those of neoskin formed under porcine skin graft (Fig. 2).

It is shown that similar effect can be produced by cattle collagen membranes.

Our experiments demonstrated the possibility of reepithelization directly under collagen membranes. Alloskin being the most suitable membranes.

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