METHODS

Skin Repair by Transplantation of Cultured Keratinocytes

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Improvement of methods for culturing skin cells stimulates their wide application in the treatment of a variety of skin injuries. A method for restoration of the skin with cultured epithelium and cultured epithelium in combination with a derma analog (collagen gel with fibroblasts) was developed. Possible mechanisms for skin repair after transplantation of cultured allogenic skin transplants were analyzed.

Key Words: *skin repair; keratinocytes; fibroblasts; live skin equivalent*

The restoration of lost skin in diseases and injuries of different origin remains a pressing problem all over the world. It is particularly important for patients with critical and supercritical deep burns of the skin.

Treatment of trophic ulcers and nonhealing open wounds is also a serious medical and social problem.

The main method for repair of the skin is autodermoplasty based on the use of patient skin. In cases when this operation is impossible because of grave status of the victim or deficit of donor resources, other methods for repair of the damaged skin are required. Moreover, complications (partial or even complete graft rejection) often develop even after timely dermoplasty.

In 1970-80s, a new method for the treatment of skin defects based on the use of cultured epidermal keratinocytes was proposed. By 1989 more than 200 transplantations of cultured autologous epidermal layers were carried out around the world. We used transplantation of cultured autologous keratinocytes for the treatment of burns [1]. This method is based on physical integration of cells cultured *in vitro* into recipient tissues. Previous histological study showed that transplanted keratinocytes incorporated in the epidermis and remained there for many years [5].

Culturing of autologous keratinocytes for transplantation to prepared wound surfaces has certain limitation, the first, but not the last of which is long period (3-4 weeks) needed for preparing such a transplant. Another approach is therefore interesting: the use of allogenic keratinocyte cultured prepared beforehand and preserved (*e.g.* kryopreserved).

Thirty allotransplantations were carried out in 25 patients with burns at N. V. Sklifosovskii Institute. In one patient transplantation was carried out twice on the same skin sites. In four patients the cells were transplanted in two stages. Twenty-two transplantations on IIIB burn wounds were carried out. Complete take of epithelial layers by the first wound dressing was observed in 7 (32%) cases. Partial or complete lysis of the transplant was observed during the first dressing in 6 (27%) and 9 (41%) cases, respectively. Two allotransplantations on donor wounds and 6 on burn wounds (II and IIIA) were carried out. Complete take of the epithelial layer was observed in 100% cases by the first dressing. Complete recovery of the skin (without autodermoplasty with split skin flap) was attained in 7 (32%) cases (Table 1). It is noteworthy that in 2 patient the transplant was lyzed by the first dressing and in one patient the take was temporary. Skin repair in these three patients was due to sharply

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increased marginal epithelialization. The maximum area at which the skin was restored completely was 300 cm^2. Partial restoration of the skin was attained in 7 (32%) cases, while in 8 (36%) cases the skin was not restored.

Derma deficit is a serious problem in the treatment of deep burns. Various combinations of derma elements with cultured keratinocytes were proposed. A histotypical structure, named "live skin equivalent", was proposed at the beginning of 1980s. It is collagen gel including fibroblasts and coated with keratinocyte culture. We used a modified variant of live skin equivalent based on collagen gel microcarriers containing fibroblast and strengthened with a biodegradable network. Allogenic keratinocytes were seeded on the surface of the gel. Previously we obtained satisfactory results in the treatment of granulating wounds with transplantation of fibroblasts cultured on microcarriers [4] and in the treatment of nonhealing fistulas by using fibroblasts in collagen gel [2].

The main advantage of the proposed structure is the possibility to prepare it within 3-5 days. Another important advantage of live skin equivalent is a wide spectrum of indications for its use: treatment of wounds, burns, possibility of delivering it into body cavities, and simultaneous healing of deep three-dimensional defects of the skin. The possibility of long storage of live skin equivalent at low temperatures (below 0°C) allows its wide use in clinical practice and creation of mobilization banks for long-term storage. Pilot utilization of live skin equivalent for the treatment of deep burns gave encouraging results. Positive clinical effect was observed as early as on day 7 after transplantation of this structure with allogenic keratinocytes on wounds after deep burns, and by day 23 after the injury the skin at the site of transplantation was completely restored. Minimum duration of skin restoration by autodermoplasty in patients with deep burns is 28-30 days. In the case presented here the restoration of the skin at the adjacent sites after autodermoplasty was attained only by day 39.

It is known that cultured allogenic keratinocytes are rejected within the first 2 weeks after transplantation, though individual allogenic cells can be found in the donor epidermis at later terms. However, transplantation of allogenic layer stimulates the initiation of regenerative processes at the expense of donor cells. *In vitro* experiments also demonstrated that allogenic keratinocytes promote wound epithelialization. The role of allogenic transplants in the stimulation of wound healing is not yet quite clear. It can be hypothesized that allogenic keratinocytes produce growth factors and components of intercellular matrix for the basal membrane [9], which promotes proliferation of recipient keratinocytes and epithelialization of the wound surface. According to some authors, this transplant possesses antibacterial activity [7]. Hence, an allogenic transplant acts not only as a biological dressing, but also as a pharmacological preparation.

TABLE 1. Skin Restoration after Transplantation of Allogenic Keratinocyte Layers in Patients with Deep Burns

Degree of skin recovery	Number of cases
Complete	7 (32)
Partial	7 (32)
No recovery	8 (36)
Total	22

Note. Figures in parentheses show the percentage.

The use of allogenic cultured keratinocytes produced positive effects during the treatment of burns [8,10], chronic ulcers, epidermolysis bullosa, chronic otorrhea, Lyell's syndrome, and some other pathologies [3]. According to our data, complete or partial recovery of the skin after transplantation of allogenic epithelial layers on burn wounds was about 65% [10]. The use of allogenic cells allows repeated transplantation, if the wound is not completely epithelialized. Moreover, transplantations of allogenic and autologous keratinocytes can be combined depending on the severity of burns.

Hence, cultured allogenic and autologous keratinocytes essentially supplement the modern armory of methods for the treatment of wound defects. Their advantage is a wide spectrum of possible methodological approaches depending on the type of the wound, tasks of surgery, and patient's status. We believe that in the nearest future transplantation of human skin cells will become a routine method for the treatment of a variety of skin defects.

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