REVIEWS

Use of Cultured Fibroblasts to Restore Skin in Patients with Severe Burns

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A novel and unique method for treating skin burns by grafting cultured cells to the wound surface is described. The major graft elements are allogeneic human fibroblasts rather than keratinocytes. Experience with its use in 222 severely burned patients, including those after early surgical necrectomy, showed this method to be an effective means of treating "borderline" third-degree burns. Epithelialization times were shortened from 31±2 days to 8.4±0.9 days. When the method was combined with dermatoautoplasty using a 1:6 perforated netted skin flap, the epithelialization period decreased from 20±2.3 to 12±1.3 days. The method proved to be highly effective in the treatment of slowly healing wounds in donor areas. It is concluded that the advantages of the proposed method - high efficacy, the much lower costs (in comparison with other methods) because no expensive nutrient media or growth biostimulators have to be used, and the very short time required to obtain a graft from allogeneic fibroblasts - argue for its wide use in clinical practice.

Key Words: *fibroblast culture; treatment of the seriously ill*

Restoration of damaged skin is a major challenge in the treatment of patients with extensive and deep burns. Very effective in such situations is dermatoautoplasty with a split perforated skin flap. However, the resources of autogenous skin are not infrequently scarce. The existing methods for temporary covering of burn surfaces - with cadaveric allografts, xenografts, amnions, or synthetic materials - cannot solve these problems and do not rule out the use of autografts [13,15,24].

Back in 1941, P. V. Medowar demonstrated the possibility of growing keratinocytes *in vitro* [20]. The first reports on successful application of cultured keratinocytes for the treatment of burns appeared in 1981-82 [12,14,22]. More recently, cultured keratinocytes have begun to be used in the treatment of slowly healing donor wounds and for closing wound defects after the removal of giant nevi, tattoos, and trophic ulcers [19,25,27]. At the Ninth International Congress on Burn Trauma, held in Paris in June, 1994, more than 100 papers were presented describing the production and use of cultured cells for skin restoration and the results obtained.

In the clinical application of cultured cells, two main methods are in use. The first of these involves closure of burn surfaces with sheets of cultured keratinocytes obtained from the patient's own cells [13,16,28] or, less often, from allogeneic keratinocytes [17], while the second makes use for this purpose of so-called viable skin equivalents which include not only cultured keratinocytes but also dermal equivalents consisting of collagen, glycosaminoglycans, and a few fibroblasts [11,21].

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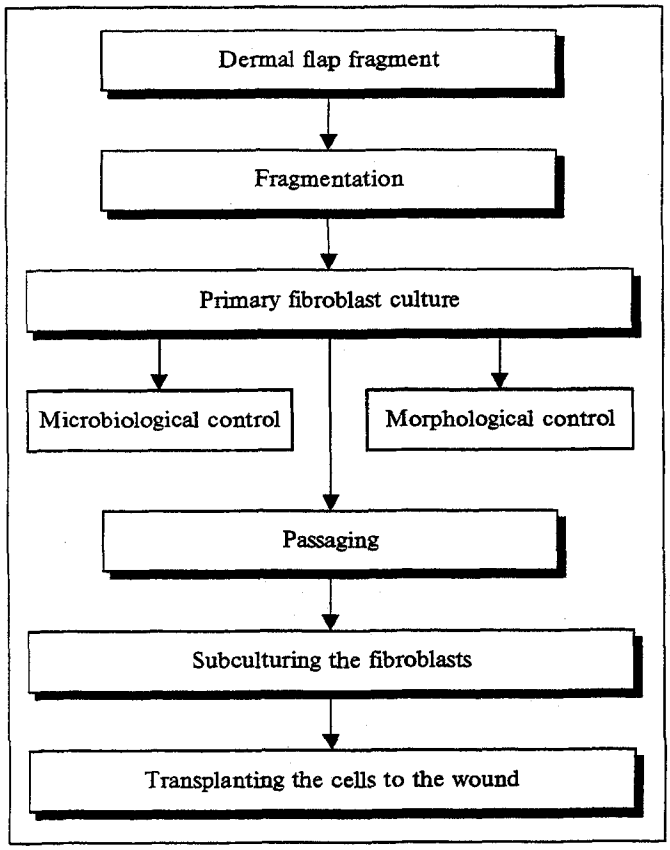

Fig. 1. Stages in the production of cultured human fibroblasts for closure of burn wounds.

However, from the very beginning, several shortcomings of cultured keratinocyte grafts became evident, precluding their wide clinical use. For one thing, it is not possible to set up a cell bank when autogenous keratinocytes are employed for grafting. For another, the period of time required to prepare a graft of sufficient size is long (3-4 weeks), and this increases the risk of burn disease in the patient and prolongs the hospital stay. Moreover, autogenous keratinocytes are very unlikely to survive if grafted to granulating burn wounds. Also, the special growth media and biologically active stimulators required for culturing keratinocytes are expensive; as a consequence, the cost of 200 cm^2 of cultured keratinocytes amounts to $13,000. Lastly, it should be stressed that the results obtained with cultured cells depend in large measure on how well the burn wound itself has been prepared for grafting. In cases where cultured cells are transplanted to the wound surface after surgical necrectomy, only 30 to 80% of the cells survive (in terms of the graft area) [16,23,26]; in cases of granulating wounds, this percentage drops to 15 [17].

Thus, as is clear from the above, the modern methods of treating burn wounds based on the use of cultured keratinocytes as the main component of the transplanted flap have serious disadvantages hampering their clinical use.

In order to overcome these disadvantages, a new, effective method of covering wound surfaces with cultured cells has been developed at the A. V. Vishnevskii Institute of Surgery, Moscow. The method differs fundamentally from all others in that fibroblasts rather than keratinocytes are used for the first time as the major components of the cultured cell sheet [2,7-10]. In developing this method, we relied on our electron microscopic and autoradiographic studies of granulation tissue, which showed that the pericytes surrounding small vessels are polypotent mesenchymal cells transforming into fibroblasts [3-6]. The latter cells exert a powerful stimulating action on the proliferation and adhesion of keratinocytes.

The use of cultured fibroblasts for closing wound surfaces has several distinct advantages over the use of cultured keratinocytes. One major advantage is that no expensive nutrient media or growth stimulators are required to obtain fibroblasts in culture, which reduces the cost of 200 cm^2 (an area equal to 1% of the body surface) to $120, i.e., by a factor of more than 100 in comparison with keratinocytes. Fibroblasts in culture are readily amenable to passaging, and since they partly lose their histocompatibility surface antigens during passaging, allogeneic cells can be utilized for preparing grafts and cell banks can be established; moreover, it takes only 2-3 days to obtain grafts suitable for clinical use instead of 3 weeks or so in the case of keratinocytes.

The fibroblasts of such grafts actively proliferate and synthesize collagen, fibronectin, and glycosaminoglycans, which become components of the extracellular matrix formed by the cells [2]. The collagen and fibronectin of this matrix stimulate both the adhesion of keratinocytes and cell proliferation [1]. Also, fibroblasts *in vitro* are essential for the differentiation of keratinocytes and for the formation of intercellular connections by the latter.

The procedure we developed for producing cultured fibroblasts is shown schematically in Fig. 1. A primary human fibroblast culture is first obtained by fragmentation of derma and its treatment with enzymes. The primary fibroblast culture is then passaged and subcultured 4 to 7 times. The last subculturing is effected on a membrane, and the resulting culture is grafted to the wound on the membrane. All fibroblast culturing is carried out in a CO_2 incubator in Eagle's medium containing 10% fetal calf serum and 2% glutamine.

Clinical studies have shown that transplantation of cultured human fibroblasts is a very effective means for local treatment of extensive "borderline" third-degree burns, of extensive thirdB-fourth-degree burns (in combination with dermatoautoplasty using a perforated 1:6 skin flap in adults and a 1:4 skin flap in children), and of slow-to-heal wounds in donor areas after dermatoautoplasty.

Cultured fibroblasts were used in our study to treat a total of 222 burned persons. The control group comprised 62 severely burned patients treated with conventional methods.

Wound healing was monitored using cytological and morphological methods (including qualitative and quantitative analyses of impression smears from wounds) and autoradiographic, electron microscopic, and histological studies of wound biopsy specimens.

The grafting of cultured fibroblasts to burn wounds was preceded by a multimodality treatment that was aimed at preparing the patient and wounds for operative intervention and included antishock therapy and treatment of burn toxemia and septicotoxemia. Local treatment involved the formation of a burn scab followed by necrectomy. A burn scab was formed, as a rule, using a 1% iodopiron solution. After necrectomy, the wounds were further prepared for grafting with modern polyethylene glycol-based ointments (laevosin, laevomecol, and 5% dioxydin) and perforated xenogeneic skin. Patients with extensive circular burns of the body and limbs were treated using a fluoridating bed. This led to rapid mummification of the burn scabs, diminished the burn intoxication, and created bacteria-free conditions for wound treatment in the pre- and postoperative periods. As a result, granulating wounds were formed whose bacterial seeding did not exceed 10^4 microbial bodies per gram. Directly before fibroblasts were grafted, the wound surface was washed with a 3% hydrogen peroxide solution. Each graft was fixed on the wound surface with paraffinized gauze. The synthetic carriers were removed 2 or 3 days after the grafting. The subsequent treatment of borderline third-degree burns was carried out using polyethylene glycol-based ointments until complete epithelialization of the wounds was achieved.

In cases of deep thirdB-fourth-degree burns, combined dermatoautoplasty with grafting of cultured fibroblasts was performed. The preparatory therapy, necrectomy, fibroblast grafting, and removal of the synthetic support were followed in adult patients by plastic closure of the wound with a 1:6 perforated netted skin flap. In children, allogeneic fibroblasts were grafted not on granulating wounds, but on the wound surface formed after excision of the burn scab shortly after trauma, followed by the performance of delayed dermoplasty with a 1:4 perforated graft. Because fibroblasts are capable of actively stimulating the adhesion and proliferation of keratinocytes, the engraftment was rapid and effective despite the high degree of autograft perforation.

The group with extensive borderline third-degree burns consisted of 27 patients aged 3 to 43 years. In this group, the area of fibroblast grafts transplanted at one time attained 900 cm^2. Complete epithelialization of the wounds closed with cultured fibroblasts occurred in 25 (92.6%) of the patients, and the mean time of wound healing was 8.4 ± 0.9 days. No epithelialization was observed in the remaining 2 patients (7.4%) with predominantly deep thirdB-degree burns. Such early epithelialization of burn wounds was never seen in the control group. The mean time of wound healing after trauma in the control patients with borderline third-degree burns was 31 ± 2 days.

The typical time course of the wound process in patients with a favorable outcome of fibroblast transplantation was as follows. Before cultured fibroblasts were grafted, the wounds were characterized by a bleeding granulated surface covered in places with fibrin. No areas of epithelialization were in evidence. Wound cytograms indicated an inflammatory or, less often, inflammatory-regenerative type of process. Microscopically, immature granulation tissue densely infiltrated by leukocytes was seen in the wounds.

On day 3 after the grafting of cultured fibroblasts, the formation of a thin, dull film was observed on the wound surface. Granulations appeared lower than in control areas and were not bleeding; the manipulations associated with wound dressing became painless. Cytological examination attested to a change from the inflammatory to a regenerative type of impression smears. Microscopically, more mature granulations with collagen fibers and much less pronounced leukocyte infiltrates were seen in the wounds.

Foci of epithelialization appeared by the end of day 7 and the formation of a squamous stratified epithelium all over the wound surface was noted by days 10-14; all of its strata were distinct, as was the basement membrane. However, dermal papillae seemed undefined. Autoradiographic and electron microscopic studies revealed high levels of DNA synthesis in basal cells of the forming epithelium and a high proliferative activity of dermal fibroblasts.

Table 1. Comparative Evaluation of the Results Obtained Using Cultured Human Cells to Treat Patients with Burns

Parameters	Keratinocytes: data reported by other authors	Fibroblasts: our data
Total No. of cases	>300 [16]	222
Burnt area, % of damaged body surface	10–85 [16,23,26]	11–75
Degree of burn	thrid–fourth [16,18,23,26]	third–fourth
Total area of grafted transplants, % of damaged body surface	0.4–59 [23,36]	0.2–55
Largest surface area of cell sheets grafted at one time, cm2	1650 [23]	3000
Graft survival rate, %	15–80 [16,17,23]	84–100
Cost (in burns involving 4% to 55% of body surface), dollars	9800–161 000 [23]	480–4000

Followup studies of this group over 18 months showed the newly formed skin areas to be in good condition.

The second group consisted of 77 adults and 18 children aged 1 to 6 years with extensive deep thirdB-fourth-degree burns.

In the adults, the burnt area comprised 30 to 70% of the body surface and the total area of grafts ranged from 500 to 5000 cm^2. In the patients with extensive deep thirdB-fourth-degree burns grafted with cultured fibroblasts in combination with their own 1:6 perforated skin, the mean time of wound epithelialization was 12±1.32 days. No lysis of the graft was observed in any of them. In the control patients, the mean engraftment time of a 1:6 perforated autodermic flap was 20±2.3 days, while the longest time of wound epithelialization was 36 days; in addition, partial lysis of the grafted autogenous skin occurred in 30% of cases.

In the children, the total burnt area comprised 5 to 80% of the body surface and the wound surface area covered with fibroblasts ranged from 30 to 3000 cm^2. Wound healing time after dermatoautoplasty in combination with cultured fibroblast grafting was 7-8 days. The reticular pattern of skin grafts was hardly noticeable, unlike in the control children. Graft survival occurred, on average, over an area equal to 84% of the area covered with fibroblasts.

The mean hospitalization time in this group was 18.5 days (22.4 days for patients with burn disease and 14.6 days for those with local burns). The mean hospitalization time of 3 children with extensive deep burns involving >50% of the body surface was 59.3 days.

The third group consisted of 38 severely burned patients with long-unhealing wounds in donor areas after their skin was taken for dermatoautoplasty. The mean period between the taking of donor skin and cell grafting (i.e., the duration of nonhealing) was 50.1±18.5 days. Fibroblast grafting to the unhealing wounds of donor areas was preceded by antibacterial therapy. Complete wound epithelialization was observed in 37 of these 38 patients by day 10 after the grafting of cultured fibroblasts; the mean epithelialization time in this group was 6.7±0.87 days *vs.* 44±4.6 days in the control patients with similar wounds who continued to be treated locally with conventional methods.

Cytological and histological studies indicated that fibroblast grafting led to a rapid succession of inflammation phases. As early as 3 or 4 days after grafting, fibroblasts were found to predominate in impression smears from the wound surface; by day 7, the proportion of fibroblasts had reached 42%. The number of polynuclear leukocytes was rapidly declining. Fibroblasts were also the predominant cell type in the granulation tissue samples from patients grafted with these cells, but not in the control group. The epidermis forming after the transplantation of cultured fibroblasts is characterized by nonuniform thickness, clear-cut differentiation of all layers, and an arrangement of cells in the basal layer in one or two rows.

Thus, we have developed a new and unique method of treating skin burns by grafting cultured cells to the wound surface. This method differs from all others available today by making use of fibroblasts rather than keratinocytes as the major graft elements.

In cases of borderline third-degree burns slow-to-heal wounds of donor areas, transplanted fibroblasts stimulate the proliferation of epidermis in its foci that remain after damage. In most cases of deep thirdB-fourth-degree burns without total damage to the skin, there is no need for the time-consuming and not always successful culturing of epidermocytes and their transplantation to the burnt skin surface. The high efficacy of the proposed method, its much lower cost because no

expensive nutrient media or growth biostimulators have to be used, and the greatly shortened period (3 days or so) required to prepare a graft from allogeneic fibroblasts argue for the wide use of this method in clinical practice.

REFERENCES

1. E. V. Glushchenko, V. P. Tumanov, G. G. Serov, and A. A. Pal'tsyn, *Byull. Eksp. Biol. Med.*, **116**, № 11, 541-544 (1993).
2. E. V. Glushchenko, A. A. Alekseev, V. P. Tumanov, and G. G. Serov, *Arkh. Pat.*, № 5, 29-34 (1994).
3. D. S. Sarkisov, B. K. Kostyuchenok, Yu. A. Amiraslanov, *et al., Byull. Eksp. Biol. Med.*, **98**, № 7, 97-99 (1984).
4. D. S. Sarkisov, A. A. Pal'tsyn, and E. G. Kolokol'chikova, *Arkh. Pat.*, № 9, 16-24 (1984).
5. D. S. Sarkisov, E. G. Kolokol'chikova, and A. A. Pal'tsyn, *Byull. Eksp. Biol. Med.*, **103**, № 6, 730-732 (1987).
6. D. S. Sarkisov, A. A. Pal'tsyn, L. I. Muzykant, *et al.*, in: *Burns and Wound Infection*, Eds. M. I. Kuzin and B. M. Kostyuchenok [in Russian], Moscow (1990), pp. 38-86.
7. D. S. Sarkisov, V. P. Tumanov, E. V. Glushchenko, *et al., Byull. Eksp. Biol. Med.*, **109**, № 3, 400-402 (1990).
8. D. S. Sarkisov, E. V. Glushchenko, V. P. Tumanov, *et al., Byull. Eksp. Biol. Med.*, **111**, № 5, 542-544 (1991).
9. D. S. Sarkisov, A. A. Alekseev, V. P. Tumanov, *et al., Khirurgiya*, № 3, 22-26 (1993).
10. V. D. Fedorov, D. S. Sarkisov, V. P. Tumanov, and E. V. Glushchenko, *Vrach*, № 11, 26-28 (1993).
11. D. Asselinean, B. Bernard, C. Bailly, *et al., J. Invest. Dermatol.*, **86**, 181-186 (1986).
12. E. Bell, H. P. Ehrlich, D. Buttle, and T. Nakatsuji, *Science*, **211**, 1052-1054 (1981).
13. E. Bell, S. Sher, B. Hull, *et al., J. Invest. Dermatol.*, **81**, № 1, 2-10 (1983).
14. J. F. Burke and I. V. Yannas, *Amer. Surg.*, **194**, 413-428 (1981).
15. I. K. Cohen, B. I. McCoy, and R. F. Diegelmann, *Ann. Plast. Surg.*, **3**, № 3, 264-272 (1979).
16. C. C. Compton, G. M. Gill, D. A. Bradford, *et al., Lab. Invest.*, **60**, № 5, 600-611 (1989).
17. M. De Luca, E. Albanese, S. Bondanza, *et al., Burns*, **15**, 303-309 (1989).
18. G. G. Gallico and N. E. O'Connor, *Clin. Plast. Surg.*, **12**, № 2, 149-157 (1985).
19. M. Koga, *Arch. Dermatol.*, **124**, № 11, 1656-1658 (1988).
20. P. B. Medowar, *Nature*, **148**, 783-784 (1941).
21. J. Nanchahal, K. Dover, W. R. Otto, and S. K. Dhital, *Lancet*, July 22, 191-193 (1989).
22. N. E. O'Connor, J. B. Mulliken, S. Banks-Schlegel, *et al., Lancet*, Jan. 10, 75-78 (1981).
23. L. W. Rue, W. G. Cioffi, W. F. McManus, *et al., J. Trauma*, **34**, № 5, 662-669 (1993).
24. R. E. Salisbury, D. W. Wilmore, P. Silverstein, *et al., Arch. Surg.*, **106**, 705-706 (1973).
25. R. Teepe, R. W. Kreis, E. J. Koebrugge, *et al., J. Trauma*, **30**, № 3, 269-275 (1990).
26. R. Teepe, E. Koebrugge, and M. Ponec, *Brit. J. Dermatol.*, **122**, 81-89 (1990).
27. J. Thivolet, M. Faure, A. Demodem, and G. Mauduit, *Bull. Acad. Nat. Med. (Paris)*, **170**, № 4, 556-562 (1986).
28. V. P. Tumanov, A. A. Pal'tsyn, and D. S. Sarkisov, *Acta Chir. Plast.*, **31**, 14-20 (1989).