The Use of Bioactive Wound Dressing, Stimulating Epithelial Regeneration of IIIa-Degree Burn Wounds

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Clinical and morphological comparison of wound healing after transplantation of living cultured allofibroblast on days 1-2 after the injury, collagen-1-based dressing with PDGF-BB, and traditional dressing with levomecol ointment showed that bioactive dressing accelerated wound epithelialization (5-7 days *vs.* 20-22 days with gauze dressing); the incidence of suppurative complications decreased, no crust formed, and epithelialization was not associated with the formation of a hypertrophic cicatrix. Biological dressing based on living cultured allofibroblasts and collagen-1 with PDGF-BB exhibited equal stimulatory effects on burn wound healing.

Key Words: *IIIa-degree burn wound; biological dressing; allofibroblasts; collagen-1; PDGF-BB*

About 500,000 patients have burn injuries in Russia annually, which necessitates search for new approaches to the treatment of burn wounds. Data on the role of stem cells (SC), cell-matrix interactions, involvement of growth factors and cytokines in reparative processes have been accumulated over the recent decade [8,10,14,16]. However, these factors do not receive due attention in local treatment of burn wounds.

According to modern concepts, two SC types are distinguished: cells of one type in the keratinocyte basal layer are responsible for the maintenance of normal epidermis structure, cells of other type in skin derivatives (hair follicles, sebaceous and sweat glands) actively repopulate the epidermis only in case of its damage [10].

IIIa-degree burn is a sort of a borderline between skin capacity to spontaneous healing and restoration of the skin integument by skin autotransplantation in IIIb-IV-degree burns. We should like to emphasize that skin derivative SC population provides spontaneous epithelialization of IIIa-degree burns.

The exceptional role of fibroblasts in skin wound healing, specifically, their migration to the focus of injury, is well known. It is hypothesized that fibroblasts migrate into the wound from intact derma and bone marrow no earlier than 5-7 days after the injury [9,15]. One of important factors initiating fibroblast migration is platelet growth factor (PDGF-BB) [13].

The team of A. V. Vishnevsky Institute, headed by D. S. Sarkisov, was the first to use transplantation of allogenic fibroblasts (AF) onto burn wound surface for stimulation of healing process; the epithelialization period was reduced to 5-7 days [5]. Morphological findings also confirmed the stimulatory effect of AF in burn wound healing [3]. However, regular use of living cultured AF for the treatment of burn wounds is difficult, requires special conditions for maintenance of fibroblast culture and preparation of these cells to transplantation. The main function of fibroblasts is the production of extracellular matrix, whose impact for wound

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process regulation and stimulation is well known [8,10]. Based on these data, exogenous collagen-1 was used for improving epithelial regeneration in a burn wound. The wound was virtually sterile on days 1-2 after burn; neutrophils, lymphocytes, and macrophages migrating into the wound not only protected it from bacterial invasion, but served as the potent source of growth factors and cytokines, inducing the development of proliferative phase of wound process [1,16]. These data also promoted the use of bioactive dressing in the treatment of IIIa-degree burns.

We evaluated the efficiency of bioactive wound dressing, stimulating epithelial regeneration of IIIa-degree wounds, by clinical morphological findings.

MATERIALS AND METHODS

A total of 95 patients with IIIa-degree burns were examined. Three groups of patients differing by the method of local treatment were formed. The patients in the groups were of similar age (mean age 49-50 years), with similar cause of burn and burn wound area (10-60% body surface, mean area 32-35%), 70.5% men and 29.5% women. In group 1 (n=20), gauze dressing with levomecol ointment was used, in group 2 (n=40), AF transplantation, and in group 3 (n=35), dressing based on collagen-1 with PDGF-BB were used (Table 1).

Human fetal AF (strain M-22) were a kind gift from M. P. Chumakov Institute of Poliomyelitis and Viral Encephalitides. Clinical studies were approved by a decision of the Academic Council of N. V. Sklifosovsky Institute. The cells were prepared for transplantation at Laboratory of Tissue Culture of N. V. Sklifosovsky Institute in accordance with methodological recommendations of Ministry of Health of the Russian Federation [4]. A total of 10.742 m^2 dressing with an AF monolayer were used for the treatment of 40 patients.

Biological dressing with collagen was made using collagen-1, isolated from human tendons by extraction in 0.1 M acetic acid by a previously described method [6]. The two-layer dressing used for the treatment consisted from a thin (no more than 1 mm) sponge from lyophilized collagen and a sublayer of carboxyl-P (polysilaxan/polycarbonate film, perforated for exudation discharge). This polymeric film was chosen not only for providing the integrity of the collagen sponge, but also for maintaining adequate gas exchange and humidity in the wound. Fresh defrosted donor serum with AB(IV)Rh-Kell$^-$ phenotype served as the source of PDGF-BB. Collagen sponges were impregnated with this serum for PDGF-BB protection from destruction by proteases present in the wound exudate and for providing gradual release of the factor into the wound environment. A total of 10 m^2 dressing with collagen was used in 35 patients.

Primary debridement of burn wounds, which were then dressed, were carried out under conditions of dressing room of Burn Center during the first 2 days after the trauma.

Polymeric films with cells or collagen were fixed on humid drying wounds with gauze. The next dressing was carried out after 2-3 days.

Epithelial regeneration process was controlled morphologically. Wound tissue specimens (5×5 mm) were collected (under local anesthesia) for histological examination before dressing and 3, 5, and 7 days after application of dressing materials. Wound biopsy specimens were fixed in 10% neutral formalin, the sections were stained with hematoxylin and eosin and with picrofuchsin after Van-Gieson.

RESULTS

The morphology of wound caused by IIIa-degree burn was characterized by the absence of epidermis, destruction of the dermal papillary layer with pronounced leukocytic infiltration and destructive changes in the reticular layer (Fig. 1). Hair follicles, sebaceous and sweat glands in the depth of the reticular layer remained intact.

Regeneration of the papillary layer of the derma, in which vessels were seen, with fibroblasts predominating among the cells, was observed 3 days after application of biological dressing based on collagen-1 with PDGF-BB. Edema and destructive changes in the papillary layer persisted; active cellular reaction was noted around the glands. Small foci of the neoepithelium formed over the entire surface of the wound (Fig. 2). Vessels and fibroblasts were seen in regenerated papillary layer of the derma; however, the papillary layer was not restored on the wound site healing under gauze dressing (Fig. 3). On day 7, the wound was completely epithelialized under biological dressing, while just focal epithelialization was seen on the wound site without it (Fig. 4). Slight small local suppurative foci at the interface with deep burns were observed in 3 (8.5%) patients from group 3.

After transplantation of AF on days 1-2 after the injury, the wounds epithelialized by days 5-7 (Table 1). Crusts formed in 5 (8%) patients, local suppuration was observed in 4 (10%) cases. In the group of patients with wounds dressed by gauze, epithelialization was observed on days 20-22, crusts formed in 16 (80%) patients, pyonecrotic complications developed in 7 (35%) patients.

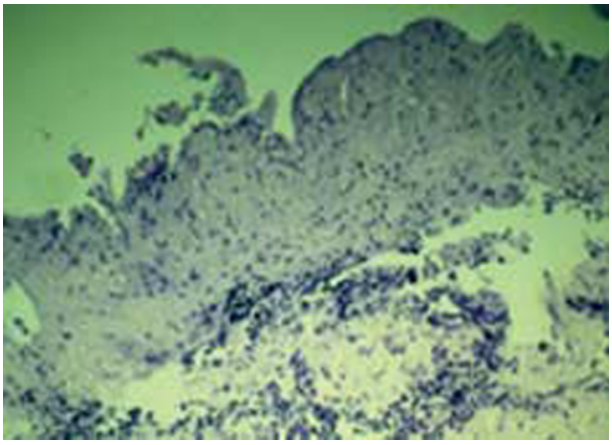

Fig. 1. Burn, IIIa degree (day 2): no epidermis, destruction of papillary layer, pronounced leukocytic infiltration, destructive changes in dermal reticular layer. Here and in Fig. 2, 3: hematoxylin and eosin staining (×200).

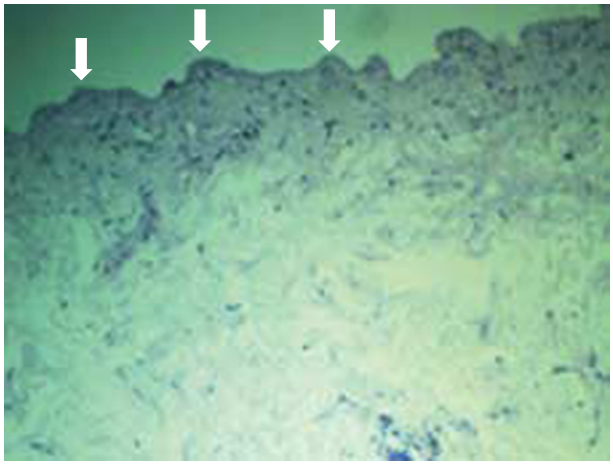

Fig. 2. Day 3 after application of dressing with collagen-1 and PDGF-BB: vessels and fibroblasts in restored dermal papillary layer. Arrows show foci of neoepithelium.

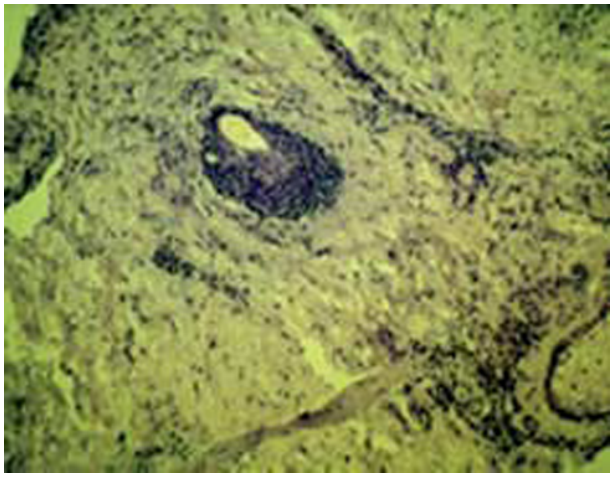

Fig. 3. Burn wound on day 3 after gauze dressing.

The use of AF transplantation and collagen-1-based biological dressing with PDGF-BB led to rapid epithelialization of the wounds, even over the entire wound surface, in contrast to marginal and focal epithelialization in traditional treatment. Suppuration of the wounds under biological dressing was local. On the whole, these complications were negligible and virtually did not modify the rate and type of epithelialization of the major area of wound surface. Epithelialization of the wounds in these two groups was free from scars, while treatment with the use of gauze dressing was often associated with the formation of hypertrophic scars. By days 10-14 signs of pigmentation recovery were noted in the neoepithelium, particularly demonstrative in the yellow race representatives; the first hairs appeared. Delayed results (1.5-2.0 months after discharge) showed that the epithelial status, color, and elasticity corresponded to those of normal skin; no signs of delayed hypertrophy were detected. The patients had no complaints.

Hence, the stimulating effects of living cultured fibroblasts and collagen-1 with PDGF-BB were virtually equal. The use of cell culture and collagen dressing for healing of IIIa-degree burn wounds at later periods after the injury, when cellular composition of the wound and biochemical composition of its microenvironment are qualitatively different, is less effective because of higher probability of pyonecrotic complications. Application of biological dressing is not indicated forAases with insemination of wound surface higher than 10^4 bacterial bodies/g tissue.

The following clinical observation demonstrates the efficiency of collagen-1-based biological dressing with PDGF-BB.

Patient O., 37 years, body weight 160 kg. Diagnosis: II-degree burn of 5% body surface inflicted by boiling water and IIIa-degree burn of 10% body surface. Fourteen hours after the injury debridement of burn wounds (Fig. 5, *a*) was carried out and dressing with collagen-1 and PDGF-BB was applied. After 3 days, a thin layer of the neoepidermis formed over the entire wound surface (Fig. 5, *b*). On day 7 after the injury, full-value epithelialization over the entire wound surface was seen at sites of collagen dressing application. No signs of epithelial hypertrophy were noted. The patient was discharged in a satisfactory state on day 9 after the injury (Fig. 5, *c*).

Distribution of patients, hospitalized at Burn Center of N. V. Sklifosovsky Institute in 2003-2005 by the severity of burns, indicated that 70.3% patients had II-IIIa-degree surface burns [2]. In one-third of these patients burn injuries occupied more

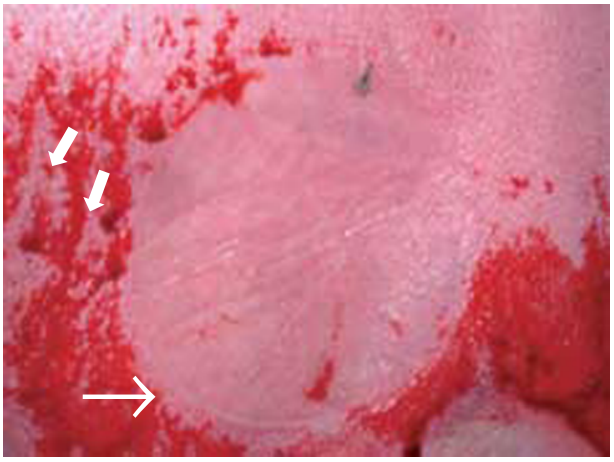

Fig. 4. Wound epithelialization under collagen-1-based dressing with PDGF-BB on day 7: marginal epithelialization at the interface of dressing (long arrow) and focal epithelialization (short arrows) near the dressing.

than 20% body surface area and the wound process was associated with shock and burn disease, which augmented the injury, led to development of pyonecrotic complications and prolongation of wound healing. Moreover, extensive IIIa-degree burns of the skin as a rule heal with a hypertrophic cicatrix, leading to disability, this fact once more indicating the importance of the problem of treatment of subdermal burn wounds.

Regeneration of the epidermis in II-degree burn is determined by resident SC in retained keratinocyte basal laye, and the epithelial multilamellar structure is completely restored by days 7-14, while in deeper burns the regeneration of the epidermis depends solely on SC of the skin derivatives (hairy follicles, sebaceous and sweat glands) [12]. Hence, the severity of skin burn determines the involvement of different SC populations in regeneration of the epidermis. The appearance of hairs in the new epithelium presumably indicates activation of the hair follicle multipotent SC, forming the base for the epidermis and hair [7].

Matrix molecules, in addition to obvious fixation of cells, dynamically regulate the key functions of the cells (proliferation, differentiation, movement), while its composition, in turn, is stringently regulated by cells [12,14]. Fibroblasts provide a certain balance of the processes of synthesis, secretion, and degradation of extracellular matrix molecules in the derma, and particularly of collagen-1 molecules [10] determining its structure and functions. However, no fibroblasts are present in the subdermal burn wound during the first two days after the injury; they migrate to the wound later [9]. Platelet dermal growth factor promotes rapid attraction of fibroblasts from intact derma to the focus of burn trauma [13]. On day 1 after the injury, the level of PDGF-BB in the burn wound exudate is very high [16], but it rapidly decreases, and therefore prolonged release of this factor from collagen sponge promotes attraction of fibroblasts from intact tissues adjacent to the wound.

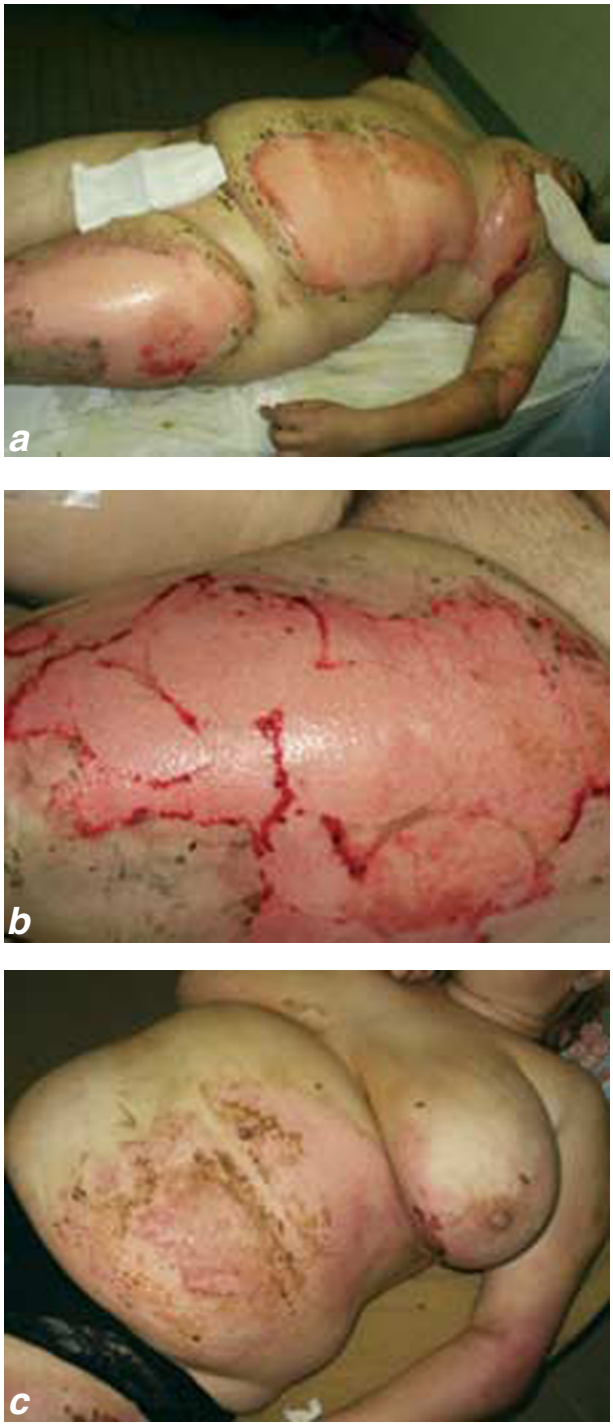

Fig. 5. Patient O. Time course of epithelialization of II- and IIIa-degree burn wounds. a) status on admission to hospital 14 h after injury; b) day 3 after collagen dressing application; c) day 9 after injury.

TABLE 1. Comparative Evaluation of Efficiencies of Dressing of Different Kinds on Healing of IIIa-Degree Burns

Parameter	Dressing type		
	gauze impregnated with levomecol ointment (group 1)	based on living AF (group 2)	based on collagen-1 and PDGF-BB (group 3)
Number of patients	20	40	35
Age, years	29-70 (49±2)	27-71 (49±1)	26-74 (50±3)
Area of injury, % of body surface	10-55 (32±3)	10-60 (35±6)	10-60 (35±5)
Day of epithelialization	20-22	5-7	5-7
Crust formation	16	5	2
Suppurative complications	7	4	3
Epithelialization type	Hypertrophic cicatrix	No cicatrices	No cicatrices

Hence, pathogenetically justified local therapy of IIIa-degree burns consisted in the use of living cultured AF or dressing with a thin collagen sponge with PDGF-BB. Both dressing types were equally effective. The biological collagen dressing with donor serum is preferable, because it is easier available, can be stored for a long time, and is economic.

REFERENCES

1. I. V. Voronkina, N. V. Kalmykova, and G. P. Pinaev, *Tsitologiya*, **46**, No. 4, 361-375 (2004).
2. A. S. Ermolov, S. V. Smirnov, V. B. Khvatov, *et al.*, *Skoraya Pomoshch'*, No. 3, 171-172 (2006).
3. E. G. Kolokolchikova, L. I. Budkevich, A. E. Bobrovnikov, *et al.*, *Byull. Eksp. Biol. Med.*, **131**, No. 1, 107-111 (2001).
4. Methodological Recommendations of Ministry of Health of the Russian Federation *"Method for Surgical Treatment of Burns in Children by Using Cultured Human Allofibroblasts"*, Moscow (1997).
5. D. S. Sarkisov, A. A. Alekseev, V. P. Tumanov, *et al.*, *New Methods for Treatment of Burns Using Cultured Allofibroblasts* [in Russian], Saratov (1998), p. 31.
6. M. Z. Aberdin and R. Riemschneider, *Die Angewandte Makromolekulare Chemie*, **11**, No. 1701, 107-122 (1983).
7. L. Alonso and E. Fuchs, *PNAS*, **100**, Suppl. 1, 11,830-11,835 (2003).
8. R. A. Clark, *J. Dermatol. Surg. Oncol.*, **19**, No. 8, 693-706 (1993).
9. A. el-Ghalbzouri, S. Gibbs, E. Lamme, *et al.*, *Brit. J. Dermatol.*, **147**, No. 2, 230-243 (2002).
10. A. K. Ghosh, *Exp. Biol. Med.*, **227**, No. 5, 301-314 (2002).
11. M. Ito, Y. Liu, Z. Yang, *et al.*, *Nature Med.*, No. 11, 1351-1354 (2005).
12. D. A. Lauffenburger and A. F. Horwitz, *Cell*, **84**, No. 3, 359-369 (1996).
13. W. Li, J. Fan, M. Chen, *et al.*, *Mol. Biol. Cell*, **15**, No. 1, 294-309 (2004).
14. P. Libby and R. T. Lee, *Circulation*, **102**, 1874-1876 (2000).
15. C. F. Roufosse, N. C. Direkze, W. R. Otto, and N. A. Wright, *Inter. J. Biochem. Cell. Biol.*, **36**, 585-597 (2004).
16. S. Werner and R. Grose, *Physiol. Rev.*, **83**, 835-870 (2003).