

Effect of Allofibroblast Transplantation on the Wound Process and Its Outcomes in Burned Patients

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Clinical and morphological studies of the effects of allofibroblast transplantation on the wound process were carried out in patients with extensive deep burns. Transplantation of allofibroblast culture stimulated the reparation process, promoted rapid increase in the counts of fibroblasts and functionally active macrophages, formation of normal granulation tissue, and early effective autodermoplasty. Transplantation reduced the number of operations and treatment duration and costs and ensured better esthetic results.

Key Words: *burns; allofibroblasts; wound process; morphology*

One of the main problems in the treatment of patients with extensive burns is effective repair of skin integrity, particularly in cases with burns involving more than 30%, because the potentialities of traditional autodermoplasty are limited by the area of donor surfaces [7]. The problem is aggravated in cases with so-called borderline or mosaic 3rd degree injuries, when radical removal of dead tissues is impossible or when the bottom of the wound after necrectomy is presented by subcutaneous fat [2]. Provisional biological coatings (alloskin, xenoskin) and cell technologies are recommended in these burns [1,5,6,8]. A method for culturing and indications to transplantation of allofibroblasts in extensive burns have been developed at A. V. Vishnevsky Institute of Surgery in 1993 [4]. The efficiency of this method for the treatment of burn wounds was confirmed by the results of analysis of changes in the impression smears from the wounds after transplantation of cultured fibroblasts, by humoral and cellular immunity values, and bacteriological control of the wounds [3]. This study presents the results of morphological analysis of burn wound biopsy specimens treated using allofibroblasts proving the effects of allogenic cells on the wound process.

MATERIALS AND METHODS

The role of allofibroblasts in the wound process in patients with burns was evaluated by the results of analysis of examinations and surgical treatment of 138 victims with extensive deep burns, treated at Department of Thermal Injuries and Plastic Surgery, V. K. Gusak Institute of Reparative Surgery, Academy of Medical Sciences of Ukraine, in 2005-2006. The patients were divided into 2 groups. The main group consisted of 99 patients transplanted allofibroblast cultures. The reference group consisted of 39 patients in whom necrectomy was carried out without transplantation of cell cultures. The patients in the two groups were similar by age, sex, etiology, area and depth of tissue injury, severity of burn disease, periods of hospitalization, location of injuries, and distribution of communal and occupational injuries ($t < 2$; $p > 0.05$).

Both groups consisted of men aged 17-70 years (mean age in the main group 44.54 ± 2.60 years, in the reference group 43.00 ± 2.51 years). The group of victims with burns inflicted by the flame was most numerous (46.37% in the main group and 42.39% in the reference group).

The treatment strategy used in inpatient setting for victims with burns is early surgery. Early necrectomy was carried out on day 4.16 ± 0.66 in the study group and on day 5.49 ± 1.40 in the reference group.

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A total of 539 operations were carried out in 138 patients.

The object of the study was the course of the wound process after removal of necrotic tissues in patients with 3rd degree burns, when the wound bottom after necrectomy was presented by subcutaneous fat and the result of radical removal of necrotic tissue was doubtful. This wound status precludes simultaneous autodermplasty because of high probability of lysis of the transplanted autografts.

The wound process was stimulated by cultured fibroblasts in patients included in the study (main) group. Allofibroblasts were transplanted onto the wound surface after early necrectomy. In controls, the wounds were dressed with gauze dressing after necrectomy. Wound dressing was carried out by the standard protocols in both groups.

Comparative morphological study of biopsy specimens from burn wounds of patients from both groups was carried out using common histological, histochemical, and immunohistochemical methods in order to objectively prove the positive effect of allofibroblasts on the wound process.

RESULTS

Free combined autodermplasty was carried out earlier in the main group than in the reference group (on days 3 and 6, respectively). The periods between the operations were reduced significantly in the main vs. the reference groups: 2.2 times between the first and second operations, 1.3 times between the second and third and between the third and fourth operations. As a result, patients of the main group stayed in hospital during 57.69 ± 1.13 days vs. 63.33 ± 2.32 days in the reference group ($p < 0.05$, $t = 2.2$).

Histological picture of the wounds after necrectomy was similar in both groups. Necrosis was an obligatory component of the morphological picture in all cases. The thickness of the necrotic layer varied greatly, involving all layers of the skin in some places. The edges and surface layers of the wounds were presented by tissue and cell detritus, neutrophilic polymorphonuclear leukocytes, and lysed erythrocytes (surface leukocytic necrotic layer). Porous necrosis of subcutaneous fat, stasis, extravasate, and microclots in the lumens of blood capillaries were seen in deeper layers of the wound. Edema and detachment of the epidermis with the formation of large cavities were seen in sites with retained epidermis in the wound edges (Fig. 1).

On day 10 after necrectomy and local therapy of the wounds without allofibroblast transplantation, a wide compact leukocytic necrotic layer containing damaged cells of local tissue and fibrin layers was

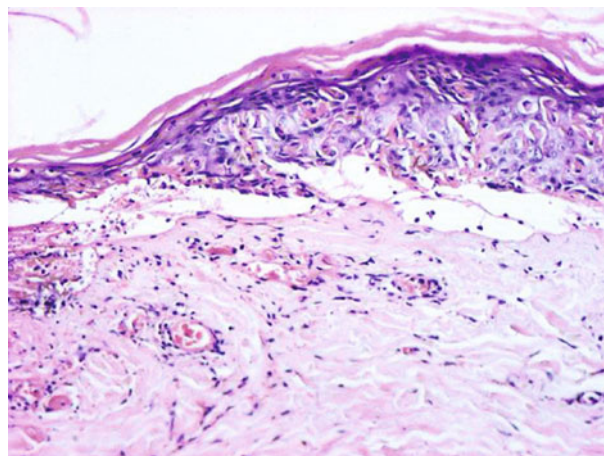


Fig. 1. Detachment of the epidermis in the wound edges, plethora, capillary thrombosis, lymphocyte infiltration. Hematoxylin and eosin staining ($\times 75$).

retained in the surface part of the wound. Despite pronounced perifocal vascular reaction, the vascular lumen in the surface compartments contained mainly erythrocytes and solitary leukocytes. Leukodiapedesis was slight during this period. The majority of neutrophilic polymorphonuclear leukocytes of the leukocytic necrotic layer were in a state of leukoplasia (Fig. 2).

The study of the deep layers of these wounds during the same period showed the formation of pronounced inflammatory infiltration. The infiltrate was mainly presented by segmented neutrophils with few lymphocytes and macrophages.

On day 12 after necrectomy and continuing local therapy, the neutrophilic infiltration stabilized; the counts of macrophages and young fibroblasts increased. Granulation tissue was forming during this period. The walls of new vessels were thick, edematous, impregnated with plasma; PAS reaction was positive. Thrombosis of some capillaries with perifocal slight edema

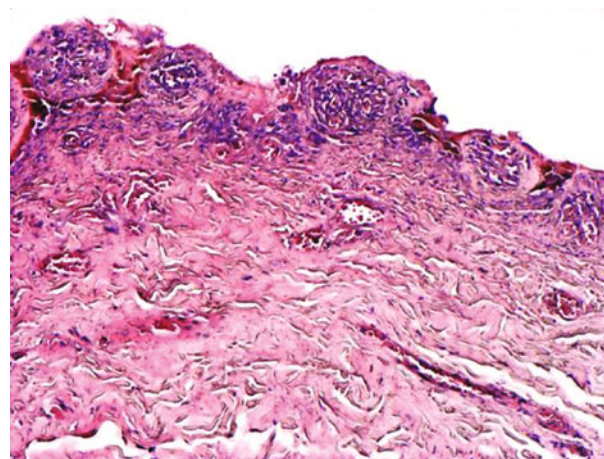


Fig. 2. Burn wound on day 10 after early necrectomy without allofibroblast transplantation. Hematoxylin and eosin staining ($\times 60$).

was detected in some sites. Despite a significant increase in the specific volume of mixed neutrophilic and mononuclear infiltration from the beginning of treatment until day 14, the count of active phagocytes (including polymorphonuclear leukocytes with intact cytological structure) and macrophages was low.

The formation of defective granulation tissue, characterized by pronounced edema, neutrophilic and lymphoid infiltration, scanty fibroblasts, and productive desquamation vasculitis, was observed in one-third of the reference group. Drastic swelling and destruction of the endothelium, thickening and stratification of basal membranes (leading to disorders in transcapillary exchange), plasmatic imbibition and neutrophilic infiltration of vascular walls were paralleled by sludge phenomenon, erythrocyte aggregation and agglutination with the formation of hyaline, fibrin, or platelet clots. This resulted in high permeability of the vessels and lymphostasis. The granulation tissue contained scanty fibroblasts with PAS-positive cytoplasm, porous plasma, and lymphoid cell infiltration.

The macrophage-fibroblast and fibroblast-vascular interrelationships were studied in biopsy specimens from these patients. High specific volume of vessels and fibroblasts in sites of granulation tissue maturation did not correspond to the formation of collagen fibers. Collagen fibers in these sites were slightly PAS-positive, mainly picrinophilic (van Gieson staining).

An important feature of the reparation process in this group was alternation of loose and compact fibrous connective tissue separated by infiltrate with lymphoid and solitary plasmatic cells in the wound.

Pronounced polymorphism of capillaries indicated discoordinated development of the reparative reaction and dysregulation of the reparative processes.

Hence, early necrectomy and subsequent therapy without allofibroblast application was followed by gradual reduction of the inflammatory process and formation of coarse connective tissue on days 12-14 postoperation. The formation of defective granulation tissue was observed in one-third of patients.

Positive changes were observed in biopsy specimens from patients of the main group (transplantation of fetal allofibroblast culture). The intensity of inflammation decreased, while reparation was stimulated similarly to that in the control group. However, the rate of inflammatory process reduction in the patients transplanted fetal allofibroblast culture was higher than in the controls. This can be explained by two facts, associated with the use of allogenic fibroblast culture: 1) as a provisional biological coating, the culture protected the wounds from drying and created optimal conditions for granulation tissue growth and 2) fibroblasts stimulated wound healing by releasing bioactive substances and growth factors.

Starting from day 2 after necrectomy with transplantation of allofibroblast culture, the count of polymorphonuclear leukocytes decreased significantly virtually in all histological preparations; no morphological signs of new portions of leukocytes in the wound were detected on day 10. No leukodiapedesis, no leukocytes with the initial signs of degradation, a significantly lesser leukocytic infiltration in all layers of the wound than in the control group were observed (Fig. 3).

A very important prognostic sign, indicating effective development of reparation, was fibroblast proliferation, first perivascularly, then at sites adjacent to the transplanted fibroblast culture. Growth of young granulation tissue with numerous vessels was observed at rather large areas.

The formation of vessels was observed in sites of more mature connective tissue. Pericytes proliferated around these vessels, these cells containing intermediate actin and vimentin filaments. Similar intermediate filaments were detected in adjacent fibroblasts (that is, these cells had the structure characteristic of myofibroblasts). Effective autodermoplasty can be carried out under this conditions.

Hence, favorable time course of reparative processes was observed in biopsy specimens from burn wounds of patients receiving combined treatment including transplantation of allofibroblast cultures.

Early surgical treatment of extensive deep burns in combination with transplantation of cultured allofibroblasts had a favorable impact on the wound process. Neutrophilic infiltration decreased, as did tissue edema and microcirculatory changes. In addition, transplantation of allofibroblasts stimulated the reparation process: the counts of fibroblasts, functionally active macrophages rapidly increased, normal granulation tissue was forming. All these events al-

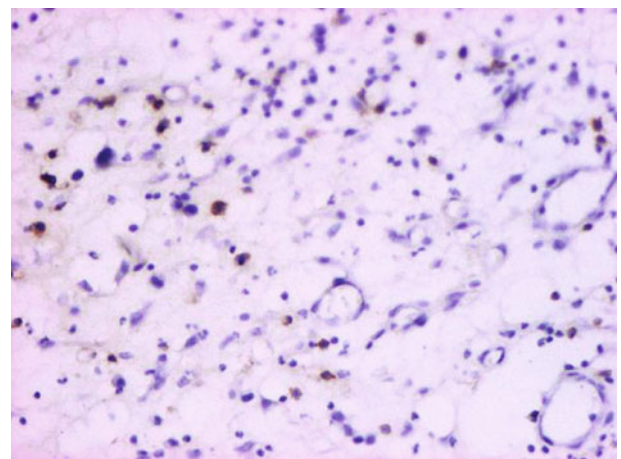


Fig. 3. Burn wound on day 10 after necrectomy in patients of the main group. Minimum leukocytic infiltration with solitary lymphoid elements in the wound. Immunohistochemistry with antiCD8 monoclonal antibodies ($\times 150$).

lowed early effective autodermoplasty and reduction of the number of surgical interventions, duration of treatment, and treatment costs. Rapid wound healing prevented the formation of coarse postburn cicatrices and promoted the improvement of esthetic results of treatment.

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