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Original article

Reprint

Pathomorphological characteristics of the wound bed prior to skin autografting

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Abstract: Objective: to conduct a comparative pathomorphological analysis of wounds of various origins requiring full-thickness skin autografting.
Materials and Methods. Histomorphological comparison of the wound bed prior to plastic surgery with full-thickness skin autografts was performed in three groups of patients: (1) during excision of scar tissue in elective surgery; (2) in case of traumatic skin detachments with autografting sensu Krasovitov; (3) when excising the granulation tissue to the fibrous layer. The object of the study included biopsy specimens from patients of three study groups.
Results. The histological picture of wounds after removal of scars was characterized by well-developed dense fibrocellular connective tissue and had signs of chronic inflammation. In contrast to the cicatricial wound, acute lesions were characterized by granulation and mature dense fibrous connective tissues with pronounced inflammatory changes, each of which had its own characteristics.
Conclusion. The results of the comparative analysis revealed the features of the morphological picture of wounds depending on the type of damage. In the group of acute injuries, traumatic and burn wounds, the most pronounced tissue damage was revealed. Given the obtained data, it should be assumed that full-thickness skin autografting will yield the best result in the group of patients after the planned excision of scar tissue.

Keywords: burn, granulating wound, skin detachment, skin autografting.

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Introduction

Burn injuries are among the most important medical and social problems of modern society, due to their prevalence, high patient mortality, substantial temporary labor losses, and primary disability. Worldwide, burns are the fourth most common type of injury. According to the World Health Organization, nearly six million people seek medical help with burns annually [1-3]. In the surgical tactics of active treatment of burn wounds, their timely closure is of the utmost importance. The success of full-thickness skin autografting largely depends on the timing of the operation. Despite numerous studies of wound healing processes after burns, the issues of repair and angiogenesis in autografts remain insufficiently studied [4-6]. The main type of operations to restore the integrity of the skin in patients with burns is dermatome skin autoplasty with a split graft. However, the results of plastic surgery with a full-thickness skin autograft are as close as possible to healthy skin. Graft engraftment depends on the level of wound bed vascularization and the degree of its infection. Unfavorable local factors are exposed loose subcutaneous adipose tissue, tendons, insufficient hemostasis, incomplete removal of necrosis, and inflammation in the wound due to late surgery after the injury [7–9]. As a result, the morphological study of the burn wound bed at different periods of full-thickness skin autografting is quite relevant.

June 26, 2020, marks the 85th anniversary of the first free skin graft for traumatic skin detachment using the method of Vladimir K. Krasovitov. This grafting technique is used all over the world and bears the name of the author. VC. Krasovitov was the founder of the plastic surgery school in the Kuban region. In the 21st century in this region, methods of full-thickness plastics continued improving, not only in clean surgical conditions, but also transplantation to granulation tissue 2-4 weeks after injury. The main condition for engraftment on a granulating (purulent) wound is the excision of the granulation tissue to the lower fibrous layer [10].

A full-thickness skin graft is close in its qualities to healthy skin, it scars less than a split graft. At the same time,

with full-thickness skin grafting, a defect occurs in the donor site, on which grafting is performed with split grafts or, in small areas, primary sutures are applied. These technical limitations are indications for surgical treatment in cosmetic and functional areas, in small areas of the body [11].

The development of surgical techniques for grafting with a full-thickness autograft on a purulent wound and the study of the wound bed during engraftment of a full-thickness graft remains relevant at present, since it allows predicting the course of the wound healing process and achieving optimal functional and cosmetic results.

Objective – to conduct a comparative pathomorphological analysis of wounds of various origins that require full-thickness skin autoplasty.

Materials and Methods

A histological and morphological comparison of the wound bed prior to grafting with full-thickness skin autografts was carried out in three groups of patients: (1) with excision of scar tissue in elective surgery (n=10); (2) with traumatic skin detachments with autografting sensu Krasovitov (n=8); (3) when excising the granulation tissue to the fibrous layer (n=13). Histological material for the study was obtained within five days after the operation.

Tissue fragments (wound bed) were excised under general anesthesia during surgical treatment before grafting with full-thickness autografts.

All biopsy samples were subjected to standard histological processing, including pre-fixation of tissue fragments in 10% buffered formalin solution for 18-24 hours, histological fixation in isopropyl alcohol using an STP-120 Microm histological processor, and filling biopsy specimens with Histomix in plastic cassettes using a Microm filling station. Cutting of the material with the preparation of thin paraffin sections was carried out with a rotary microtome Microm E-340n. Staining of the preparations was conducted according to the standard method with hematoxylin–eosin and picro-fuchsine staining sensu Van Gieson. Immunohistochemical studies included staining preparations with anti-CD3, anti-CD68 and anti-CD31 antibodies, as well as antibodies to epidermal cytokeratin AE1/AE3 and type IV collagen. Microscopic examination was performed using an Olympus IX51 microscope with an Olympus CellSense imaging system at ×40 and ×200 magnifications. For computer morphometry, the ImageJ software was used. To count cells and determine linear dimensions, computer morphometry was performed using the ImageJ sofrware and the IHC metrics add-on (National Institutions of Health, USA). To calculate the number of cells, we used the analytical algorithm developed by the authors, which included obtaining a digital image of tissue structures, determining the total area of the section using the Manual Selection tool, setting the Particle Analyzer tool (setting the size and nature of the relative positions of desired objects), determining the total number of the desired cellular elements in the biopsy sample using the particle analyzer [12]. For morphometry of preparations after immunohistochemical reactions, we employed the IHC metrics tool, in accordance with the user manual.

Results

Histological preparations from the group of patients who underwent elective scar removal were characterized overall by well-developed dense fibrocellular connective tissue (*Figure 1*). In most preparations, the fibrous component predominated, collagen fibers were not fragmented, but were arranged randomly. The thickness of the connective tissue layer averaged 1.5 mm. There were isolated cases of the formation of numerous small foci of stratified epithelium inside the connective tissue (epidermal cell differentiation was confirmed by immunohistochemical studies with antibodies to cytokeratins AE [13]), as well as isolated cases of parakeratosis.

In most preparations, a large number of small blood vessels were noted, the basement membrane of which was well visualized via immunohistochemical detection of collagen IV (*Figure 2*), as well as signs of chronic inflammation – perivascular and non-vascular diffuse and diffuse focal inflammatory infiltrates consisting of lymphocytes and macrophages (the cellular composition of the infiltrate was confirmed by immunophenotyping of CD3-positive and CD68-positive cells). However, it should be noted that the severity of the inflammatory response was generally insignificant.

Histological preparations from the group of patients with traumatic epidermal detachment were characterized by the predominance of acute injury phenomena. In most sections, hemorrhages were noted, both fresh and organizing, as well as necrotic and necrobiotic changes. They were especially pronounced in skeletal muscle tissue, which was present in a significant proportion of the studied samples (*Figure 3*).

Dense fibrous connective tissue, often containing foci of inflammatory infiltration, contained irregularly arranged collagen fibers and was mainly observed in the hypodermis.

Granulation tissue was characterized by a pronounced cellular component and significant dystrophic changes, in particular fibrinoid swelling, with damage to the walls of blood vessels. The latter was manifested by their thrombosis and hemorrhages.

In general, inflammatory infiltration was present in most histological preparations. Numerous large inflammatory infiltrates were localized in all layers of the sample tissues. Similar to group 1, the cellular composition was largely represented by lymphocytes and macrophages (determined by immunohistochemical studies with anti-CD3 and anti-CD68 antibodies [14]).

Stratified squamous epithelium was noted in half of the preparations. In most cases, the epidermis was characterized by reactive hyperkeratosis. No foci of epithelial proliferation were found inside the connective tissue (negative reaction with antibodies to cytokeratin AE).

Preparations from the group of patients with thermal injuries had in common the presence of granulation and mature dense fibrous connective tissues. The granulation tissue was characterized by the presence of a significant number of small blood vessels, most of which were plethoric. In all tissues of most histological sections, a significant diffuse focal inflammatory infiltration was noted, represented by lymphocytes and macrophages (the cellular composition was confirmed by immunohistochemical determination of CD3+ and CD68+ cells). The accumulation of inflammatory cells was mainly of a perivascular nature (*Figure 4*).

Collagen fibers of the dense fibrous connective tissue were arranged randomly, and their cellular component was weakly expressed.

In the deep layers of tissues, as a rule, at the boundary of the dermis and subcutaneous fat, significant hemorrhages were noted. Stratified squamous epithelium was absent in all histological preparations.

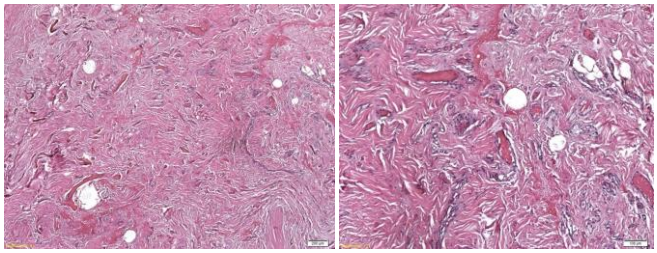

Figure 1. Sample of the wound bed in the patient after planned excision of scar tissue. The wound bed is represented by coarse fibrous connective tissue with foci of perivascular lymphatic macrophage infiltration. Staining with hematoxylin and eosin: a) magnification ×4, b) magnification ×20

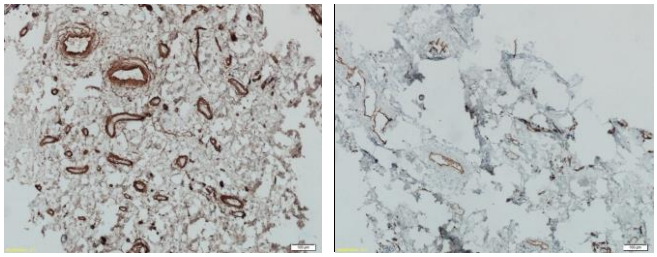

Figure 2. A patient's wound bed sample after planned excision of scar tissues: a) immunohistochemical reaction for type IV collagen revealed this substance in the basal membranes of blood vessels; b) immunohistochemical determination of platelet endothelial cell adhesion molecules CD31 revealed a positive reaction in the vascular endothelium. Magnification ×20

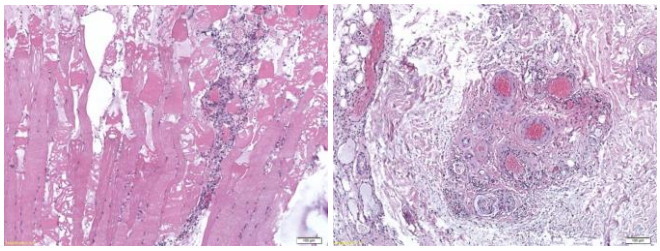

Figure 3. Tissue samples from patients after traumatic detachment of the epidermis. In some sections, ischemic muscle tissue altered by faulty nutrition with perivascular lymphatic macrophage infiltration and subfascial hemorrhage is observed; individual capillaries of the preparation are thrombosed. Staining with hematoxylin and eosin: a) magnification ×4; b) magnification ×20

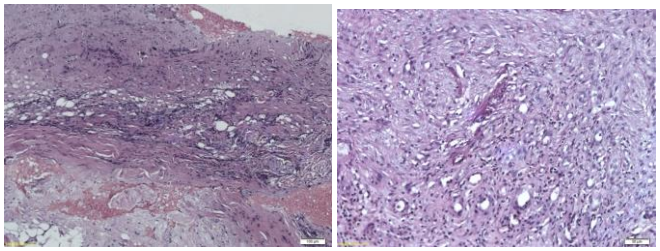

Figure 4. Patient tissue sample after thermal injury. The sample section is represented by granulation tissue with a large number of thin-walled vessels up to 20 μm in diameter and diffuse perivascular lymphatic macrophage infiltrate. Staining with hematoxylin and eosin: a) magnification ×4; b) magnification ×20

Discussion

The pathomorphological picture of the material of patients after the planned excision of scars was generally characterized by the predominance of coarse fibrous connective tissue with a disordered arrangement of collagen fibers, as well as the presence of a large amount of granulation tissue with a well-defined cellular component and a large number of vessels. Inflammatory changes were chronic and minimally expressed. This finding could be explained by the low contamination of the surgical wound. In a number of preparations, the development of the epidermis with preserved stratification was noted. Considering the presence of cell clusters of epithelial origin, the source of which, apparently, was hair follicles, we could assume the presence of a significant amount of epidermal growth factor in the tissue. Together with low microbial contamination, as well as a well-developed microcirculatory bed, this created good conditions for complete regeneration of the skin area and engraftment of the dermal autograft [15].

A group of tissue samples after traumatic detachment of the epidermis was characterized by pronounced changes inherent in acute injury: necrosis, dystrophy, edema, and tissue fragmentation. Besides, in the preparations of this group, inflammatory infiltration was more pronounced than in the group of cicatricial lesions. However, a substantial share of this group preparations contained the epidermis, apparently intact after direct injury and not related to the regeneration process at the time of the study [16].

The group of samples after burn injuries was characterized by significant damage to blood vessels, which was reflected in numerous hemorrhages in different layers of tissues, as well as in plethoric capillaries and arterioles [17]. Significantly pronounced were also inflammatory changes identified in virtually all biopsy specimens. Such profound changes could be explained by degeneration of the blood vessel walls in the area immediately adjacent to the lesion [18].

Conclusion

The results of the comparative analysis revealed the features of the morphological picture of wounds depending on the damage type. In the group of acute lesions, traumatic and burn wounds, the most pronounced tissue damage was identified. Given the obtained data, it should be assumed that full-thickness skin autografting will yield the best result in the group of patients after the planned excision of scar tissue.

Conflict of interest: None declared.

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