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TISSUE EQUIVALENT TRANSPLANTATION IN THE TREATMENT OF CERTAIN SKIN INJURIES

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Chronic ulcers are a common and socially significant problem worldwide. Autodermoplasty is the gold standard treatment for chronic ulcers. However, it is not always possible to perform this surgical procedure for a rather large group of patients, due to some reasons, which include high risk of autodermotransplant rejection, lack of donor material, and patient's unwillingness to undergo surgery with an often unpredictable result. A potential solution to the problem is to use skin equivalents from allogeneic donor material. The use of allogeneic (donor) human cells makes it possible to fill the deficit of the patient's donor resources and close wound without causing additional injury to the patient. This paper provides an overview of the application of foreign and domestic biomedical cell products in clinical trials and real clinical practice. We draw conclusions on the efficiency of the considered biomedical cell products in the treatment of chronic ulcers, evaluate the conducted research, and make recommendations on the most efficient use of allogeneic dermatotropic biomedical cell products.

Keywords: biomedical cell products, dermatotropic products, bioengineered constructs, skin equivalents, burns, diabetic ulcers, diabetic foot, multipotent mesenchymal stromal cells, keratinocytes.

Despite the fact that surgery has been used to deal with superficial wound epithelialization, the problem is far from being solved. This claim is illustrated by the high prevalence of chronic wounds among patients admitted for purulent surgery [1].

The purpose of this review is to summarize the well-known practice of treating skin injury, mainly chronic wounds, using products containing human living cells, called biomedical cell products (BCPs) in the Russian Federation.

First of all, it should be noted that there are certain features in the definitions of terms and concepts related to skin injuries. The terms "trophic ulcer" and "long-lasting non-healing wound" are common in Russia. A trophic ulcer can be called a dermal defect caused by a disease and lasting longer than 3 months [2]. A long-lasting non-healing wound is any wound defect resulting from an injury (including surgical injury) with a low repair rate. As can be seen, the criteria are not precise, and separation of wound types in no way affects the volume and sequence of treatment and diagnostic measures.

Most foreign researchers in their practice use exclusively the "long-lasting non-healing wound" concept; this term refers to the following pathological processes: lower limb trophic ulcers, diabetic ulcers, pressure ulcers, non-healing wounds, wounds developing at the site of an injury or surgical intervention, as well as wounds resulting from a frostbite, heat, or chemical damage to the skin [3]. According to the U.S. Food and Drug Administration (FDA), any wound that does not heal within 30 days of standard care is chronic [4].

Generalization of these processes, different in terms of cause and pathogenesis, is quite justified if they are considered in terms of clinical manifestations and processes occurring at the cellular and subcellular levels [3]. Investigating morphological changes in biopsy materials taken from chronic wounds of various types, Fedorov et al. indicated that any chronic wound (regardless of cause) is associated with similar morphological and functional disorders [5]. First of all, the functioning of cellular elements in a wound becomes pathological, extracellular matrix synthesis and remodeling processes get impaired, formation of a basement membrane and migration of keratinocytes become impossible. These changes include predominance of monocytic-macrophagal cells in the inflammatory infiltrate in combination with insufficient number of T-helper/T-suppressor cells; decreased content of type III collagen, accumulation of tenascin and plasma fibronectin, a change in the typical localization of laminin, minimal number of myofibroblasts in the

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wound defect site; changes in the mediator systems regulating the course of inflammatory-reparative response: increased accumulation of pro-inflammatory cytokines (IL-1β, MIP-lα/MIP-1β) and disruption in their normal ratio, absence of fibrogenic growth factors (TGF-β1, bFGF) in combination with increased activity of matrix metalloproteinase – 9 in the wound area [2]. There are always signs of chronic inflammation. Also, a chronic wound is characterized by low angiogenic tissue response [6], impeding neovascularization and healing.

Thus, a long-lasting non-healing or chronic wound is a dermal defect resulting from external exposure (mechanical, heat, chemical damage) or surgical intervention, existing for more than 30 days. It has a low self-healing tendency and characterized by the presence of certain changes that impede repair, creating self-sustaining system, operating like a "vicious circle". Detection of these histological and immunohistochemical signs allows to verify a chronic wound, but there are currently no clear morphological criteria for diagnosing chronic wounds.

Diabetic neurotrophic foot ulcer is one of the most typical chronic wounds [7, 8]. The non-healing nature of diabetic ulcer is explained by both abnormal inflammation phase and wound cleansing, and abnormal proliferative phase [9].

It is logical that transplantation of full-fledged skin or its elements – the intercellular matrix (hereinafter referred to as the scaffold), skin cells and dermis – can restore disrupted proliferative processes.

Autodermoplasty is the gold standard for treating chronic wounds. However, its implementation is not always possible in a sufficiently large group of patients. One of the reasons is the high risk of rejection of the autodermal graft from the wound surface, shortage of donor material, and the patient's unwillingness to undergo surgery with an often-unpredictable result. The outcomes are especially unsatisfactory in patients with neuropathic wounds amidst diabetic foot syndrome.

Various collagen dressings have been suggested as an alternative to own skin. However, they are significantly inferior in terms of effectiveness to allogeneic (taken from a donor) cell dressing material, and even more so when transplanting the skin [4]. However, collagen dressings can be used as a means of preparing for transplantation of cell structures or as a basis for creating various three-dimensional structures. Scaffolds of tissue-engineered skin substitutes are located inside the wound defect and play the role of a biological dressing, providing protection against dehydration, microorganism invasion, and toxin penetration. It is then embedded in the wound bed through natural wound healing mechanisms, such as inflammation, cellular infiltration by neutrophils, macrophages and fibroblasts, and the scaffold is neovascularized. Biocompatibility of the carrier matrix can be enhanced by adding to its structure fibroblasts that can accelerate skin regeneration processes. Dermal fibroblasts produce all the main components of the intercellular matrix (collagen, glycosaminoglycans, proteoglycans) and are also responsible for continuous matrix remodeling process. Fibroblasts are an active cellular component that can structure the dermal collagen, stimulate the growth of granulation tissue in the wound and secrete a number of growth factors promoting development of a neovascular network, formation of basement membrane and cellular migration, which accelerates skin regeneration. However, this composition leads to wound defect filling without epithelial regeneration.

Such reflections led to the creation of a heterogeneous group of products that are designed to provide complete wound closure by reconstructing the skin defect and taking over the function of the missing skin layer. An ideal skin equivalent would accelerate neoangiogenesis, extracellular matrix remodeling, granulation tissue formation and skin regeneration. The skin equivalent should lead to formation of new blood vessels and maturation of the neovascular network, resulting in decreased inflammatory process, effective healing with less scarring and wound contraction.

In the Russian Federation, some allogeneic skin equivalents are currently developed, studied under laboratory conditions, and until recently, were used in clinical practice, tested for treatment of patients with chronic non-healing wounds:

Human living skin equivalent (LSE) [10]. Ivashkin A.N. (2009) studied an LSE for the treatment of chronic wounds. The LSE is a gel-like substance composed of a synthetic mesh base. Fibroblasts are distributed in its three-dimensional structure; the surface is covered with keratinocytes [3]. LSE was used to treat patients with skin wound defects that did not heal for at least 1 month of adequate care. The patients included 22 people in the main group, and 20 in the control group. Patient groups were comparable in age, severity of accompanying conditions, initial wound defect area (29.5 ± 3.5 cm in the main group and 31.1 ± 3.6 cm in the control group). Differences in wound defect sizes did not exceed 15% within a group. The rate of decrease in the wound surface area in the main group was two times higher than in the control. Most patients with long-lasting non-healing wounds (in 81.8% of cases) underwent a single LSE plastic surgery. By the end of week 4 in the main group, healing had begun in most patients (16.7%) or there was significant decrease in wound size (41.7%). After day 25 in the control group, wound sizes decreased by less than 40%. In 20% of patients, complete epithelization did not occur at a later date. No histological and immunohistochemical verification of chronic wounds was performed before the patients were included in the study. Histological examination of wound biopsy was performed on day 5, 10, 20 of the study after treatment had started in the main and control groups. Histological signs of the onset of wound contraction process, and

granulation tissue maturation were noted in the main group on day 5. On day 10, mature granulation tissue was detected, which resembled the structure of a normal dermis, while inflammatory infiltration persisted. On day 20, biopsies of the main group showed signs of active wound reepithelization. The structure of the underlying tissue resembled a normal dermis. Inflammatory infiltration was minimal in most cases. In the biopsy specimens of the control group, such pronounced signs of healing were not observed, and inflammatory phenomena persisted for a long time. Thus, despite lack of a description of the study methodology and the small sample size, we can conclude that LSE has a significant clinical efficacy. It was studied in comparable groups with equivalent wound defect sizes, and the observed wound surface area reduction in the main group, in comparison with the control group, was confirmed by histological examination results, where the predominance of reparative processes in the LSE treatment group was clearly traced.

Dermal equivalent (two modifications: in collagen gel and based on plasma fibrin or fibrinogen). The dermal equivalent contains allogeneic dermal fibroblasts encapsulated in collagen gel. In a study by Kotslova et al. which evaluated the efficacy and safety of dermal equivalent in the treatment of wounds in patients with diabetic foot syndrome, 60 patients with granulating wounds, lasting more than 6 weeks, which did not reduce by 50% or more after 2 weeks of standard care, were included [11, 12]. The main group consisted of 40 patients; the control group had 20. All patients had superficial ulcers with no signs of infection; some patients had signs of ischemia (1A, 1C according to the University of Texas Wound Classification System). Within the groups, there was significant variation in wound defect sizes: from 1 to 25cm^2. Histological verification of chronic wounds was not performed, no histological examination of biopsy samples of wounds in the course of treatment in the groups was reported in publications. Researchers noted that ischemia significantly reduced epithelialization rate, smaller wounds healed faster, wounds more than 12 cm^2 required repeated application of the dermal equivalent within 3 to 4 months from the start of treatment. Healing was not achieved within 1.5 years in 25% of patients in the main group. This was mainly due to the lack of healing in patients with ischemia. The average complete healing timeframe in the main group ranged from 1 to 3 months (an average of 67 ± 11 days); complete healing was achieved in 65% of patients in the main group. However, it is not indicated which percentage of patients had complete wound healing 1 month after application of the dermal equivalent. The study was limited by the small sample size; the studied groups were heterogeneous in terms of wound defect sizes and number of patients; there was no description of the chosen research methodology and methods for assessing and comparing indicators in such heterogeneous groups. The average epithelialization rate in the groups after 1 and 2 months is given. The healing dynamics in the groups is not given. Thus, based on the published study, it is difficult to firmly conclude on the true effect of the dermal equivalent on wound healing.

According to Varkey et al, the following dermatotropic BCPs containing human cells were registered as of 2015 (Table 1) [14].

Table 1

List of registered dermatotropic BCPs as of 2015

Product	Composition	Comments
TransCyte®	Nylon mesh seeded with neonatal human foreskin fibroblasts that are destroyed before grafting	Temporary wound dressing upon which autografts are placed
Dermagraft®	Bioabsorbable polygalactin mesh matrix seeded with human neonatal fibroblasts and cryopreserved	Matrix facilitates re-epithelialization by the patient's own keratinocytes
Apligraf®	Bovine collagen gel seeded with neonatal foreskin fibroblasts and keratinocytes	Wound dressing with two different cell types
OrCel®	Type I collagen matrix seeded with neonatal foreskin fibroblasts and keratinocytes	Wound dressing with two different cell types
Epicel®	Sheets of autologous keratinocytes attached to petrolatum gauze support	Wound dressing with autologous cells
StrataGraft®	Full thickness skin substitute with dermal and fully differentiated epidermal layers	Made with naturally immortalized NIKS® keratinocyte cell line; contains two different cell types
Tiscover® (A-skin)	Autologous full thickness cultured skin for healing of chronic, therapy resistant wounds	Contains two different cell types
Permaderm®	Autologous tissue engineered skin consisting of epidermal and dermal cells	Contains two different cell types
denovoDerm™	Autologous dermal substitute	To be used in combination with split-thickness skin grafts
denovoSkin™	Autologous full thickness substitute consisting of dermal and epidermal layers	Contains two different cell types

It should be noted that out of 16 products containing human cells listed in these two reviews, 9 products (medical devices) are autologous, and 4 products are manufactured using cell material obtained from newborns. According to Russian law, minors cannot be cell donors in the production of BCPs, except for manufacture of autologous products.

The following skin substitutes are registered for treatment of chronic wounds: Apligraf®, Dermagraft®, TransCyte®, and OrCel®. StrataGraft® is in Phase III of clinical trials and is designed to treat severe deep burns. An FDA approval decision is expected in 2020. One cell type – allogeneic fibroblasts – contains TransCyte® and Dermagraft®. BCPs containing allogeneic keratinocytes and fibroblasts include Apligraf®, OrCel® products, which are widely and commercially available, as well as StrataGraft®, which is undergoing clinical trials.

The results of some clinical trials of BCPs are presented below.

Apligraf® (Organogenesis, USA). In a multicenter, randomized clinical trial (RCT) involving 72 patients, the outcome of using Apligraf® with standard therapy and the outcome of suing only standard therapy for the treatment of diabetic ulcers were compared. By 12 weeks, there was significant decrease in time to complete wound closure in the main group (51.5%) compared with the control (26.3%) [15]. In another multicenter, RCT involving 208 patients, who were randomly assigned to ulcer treatment either with Graftskin® (formerly Apligraf®) or saline-moistened gauze, 56% Graftskin-treated patients achieved complete wound healing compared with 38% in the control group at the 12-week follow-up visit. The Kaplan-Meier median time to complete healing was also significantly lower for Graftskin (65 days) than the 90 days observed in the control group. Osteomyelitis and lower-limb amputations were much less frequent in the experimental group [16].

Dermagraft® (Shire Regenerative Medicine, Inc., USA). A multicenter, RCT was carried out in 314 patients with chronic diabetic ulcers using Dermagraft® (main group) or conventional therapy (control group). By 12 weeks, 30.0% of Dermagraft patients had their wounds completely closed compared with 18.3% in the control group. Although the overall incidence of adverse events was similar for both groups, the number of patients who developed ulcer–related adverse events (infection, osteomyelitis, and cellulitis) was 19.0% in the Dermagraft-treated patients compared with 32.5% in the control patients [17]. A clinical study in 28 patients with chronic diabetic ulcers (longer than 6 weeks) compared Dermagraft® intervention with the control group (saline-moistened gauze alone). By week 12, 71.4% of ulcers healed in the Dermagraft group and 14.3% in the control group. Healed Dermagraft patients achieved wound closure significantly faster than the control group patients [18].

In a randomized, single-blind, clinical trial DOLCE comparing the differences between cellular-free, cellular (Dermagraft®) matrices and standard treatment for diabetic ulcers, skin substitutes showed an advantage [19]. In a Dermagraft® multicenter clinical trial, 62 patients were dressed with wet gauze or polyurethane foam bandages weekly after surgical treatment for ulcers. About 44% of patients had complete wound closure by week 12, and 52% healed by week 20. Median time to healing was 13 weeks. Dermagraft® has been shown to be safe and effective in the treatment of non-healing diabetic ulcers [20]. A multicenter RCT was performed to evaluate wound healing in 50 patients with diabetic foot ulcers. These patients were randomized into four groups (three different dosage regimens of Dermagraft® and one control group). Ulcers treated with the highest dosage of Dermagraft® healed significantly more often than those treated with conventional methods; 50% of the Dermagraft-treated and 8% of the control group healed completely [21].

TransCyte® (Shire Regenerative Medicine, Inc., USA). A clinical study using TransCyte® and silver sulfadiazine was performed with the use of paired wound sites on 14 patients. Wounds treated with TransCyte® healed much faster to a re-epithelialization state (mean 11.14 versus 18.4 days). Wound evaluations showed that at 3, 6 and 12 months, wound sites treated with TransCyte® healed with significantly less hypertrophic scarring than sites treated with silver sulfadiazine [22].

OrCel® (Forticell Bioscience, USA). To study a product for the treatment of chronic wounds, studies were conducted on patients with unhealed venous and diabetic ulcers. Clinical trials evaluating the efficacy of OrCel® for treatment of venous leg ulcers showed that 50% of OrCel® patients achieved complete wound closure at week 12 compared with 31% of subjects who received only standard therapy. Patients who received OrCel® exhibited a median time to heal of 77 days, whereas no median time was determined for the control group, since many ulcers did not epithelize completely. Results from the OrCel® pilot study in the treatment of diabetic foot ulcers show that 47% of patients in the experimental group achieved complete wound closure by week 12 compared to 23% of patients who received only standard therapy. In November 1999, OrCel® took part in a pilot study for 40 patients with diabetic foot ulcers using an updated version of the product. According to the data presented for 16 patients, it was found that at week 12, 56% of patients receiving OrCel® achieved complete wound closure, compared with 29% of patients receiving conventional care [23].

OrCel® is similar to Apligraf® because it contains both fibroblasts and keratinocytes derived from the foreskin of newborns, but uses a collagen sponge with type I collagen as matrix [24]. It is used to compensate for negative tissue defects in the wound, where it acts as a matrix for migration of the patient's own cells. In a study

that directly compared OrCel® with BiobraneTM for treating partial thickness donor wounds, OrCel®-treated areas had higher healing rates and reduced scarring. This healing improvement is due to the presence of a collagen sponge in combination with cytokines and growth factors produced by viable allogeneic cells [11].

It should be noted that when selecting clinical trials to assess their quality, the FDA chose a very limited number of trials: 95 were examined, 18 were selected [4]. Among the products considered for treatment of chronic wounds, Apligraf® and Dermagraft® studies were selected. Thier methodological quality significantly distinguished the studies of these products from the rest. Apligraf® study methodology was noted to be better than that of Dermagraft®. According to a Cochrane systematic review, Apligraf® showed a statistically significant positive effect on complete ulcer closure [25]. These data suggest that allogeneic cell products Apligraf® and Dermagraft® have most convincingly proven their effect on the healing of chronic wounds.

For the treatment of chronic wounds, Nathoo et al. recommend composite allogeneic skin substitutes in the treatment of wounds lasting more than 4–6 weeks, cell-free allogeneic skin substitutes, dermis substitutes, xenografts for other chronic wounds [26]. However, the use of bioengineered skin substitutes, according to Garwood et al., may depend on the ability of the substitute to synthesize the components of the dermis [27]. The authors distinguish dermoinductive (Apligraf®, Dermagraft®, etc.) and dermoconductive (dermo-substituting) products (Integra™ and others). From the authors' point of view, the choice of product should depend on the wound depth. For superficial wounds ending at the subcutaneous tissue, a dermo-inductive product is recommended. For injuries reaching the subcutaneous tissue and deep tissue, a dermoconductive product should be considered; if there is no neodermis formation, autodermoplasty should be contemplated.

Despite the fact that Law No. 180 FZ on Biomedical Cellular Products came into force in January 2017, until now dermatotropic BCPs were not produced on a commercial scale in the Russian Federation, and so far, there are no products approved for clinical use.

Given the number of BCPs in the world that have been gradually approved and, in some cases, withdrawn in recent years, the International Society for Cell and Gene Therapy (ISCT) has presented a brief annual report on cell products approved for clinical use in different countries [13]. The authors report that this list may not be exhaustive and that to the best of their knowledge, no cell/tissue/gene products have been authorized for marketing in Brazil, Hong Kong, Israel, Malaysia, Singapore and Taiwan as of September 2018. So, according to ISCT, as of September 2018, 6 dermatotropic products satisfying the criteria of BCPs in Russia are allowed for clinical use in the world (see table 2).

It should be noted that many products from the 2015 list are missing from the new list, and StrataGraft® BCPs are fundamentally different in composition from the 2015 version. All this indicates that the global market for BCPs, including dermatotropic ones, is still forming.

CONCLUSIONS

A study of the use of various skin equivalents suggests that BCPs have advantages in the treatment of chronic wounds. It was found that in chronic wounds, the patient's own cellular and extracellular elements are pathologically altered, and their physiological functions are impaired. In chronic wounds, the intensity of repair processes is reduced, and this therefore necessitates introduction of cellular elements from the outside, while

Table 2

List of dermatotropic BCPs approved for clinical use as of 2018

Name	Composition	Comments
JACE® (J-TEC)	Autologous cultured epidermis	For the treatment of severe burns (Japan); in the market since 2007
KeraHeal-Allo™ (KeraSkin, Biosolution Co., Ltd.)	Composite cell product – spray (allogeneic skin-derived keratinocytes suspended in a thermosensitive hydrogel)	For deep 2nd degree burns (South Korea); in the market since 2015
Kaloderm® (Tego Science, Inc)	Allogeneic keratinocytes (cell sheet)	For deep 2nd degree burn (in the market since 2005) or diabetic foot ulcer (South Korea) (in the market since 2010)
KeraHeal® (Biosolution Co., Ltd.)	Autologous keratinocytes	For deep 2nd degree burns that cover >30% of the total body surface area (TBSA) and 3rd degree burns that cover >10% of TBSA (South Korea); in the market since 2006
Holoderm® (Tego Science, Inc)	Autologous keratinocytes	For deep 2nd degree burns that cover >30% of TBSA and 3rd degree burns that cover >10% of TBSA (South Korea); in the market since 2002
StrataGraft® (Mallinckrodt plc).	Autologous skin cell product	For the treatment of deep partial thickness burns (USA); in the market since 2017

replacing the tissue mass deficit in the case of deep, full-thickness wounds.

Own skin grafting is not a reliable treatment method. It often turns out to be unsuccessful, which is unacceptable for a number of situations. Most of these patients require cellular and non-cellular elements from the outside as part of the cellular product for successful proliferation. At the same time, fibroblasts or mesenchymal stem cells should be an indispensable component of BCPs as a central element of the repair process, promoting neovasculogenesis, extracellular matrix remodeling, basement membrane synthesis, and keratinocyte migration. In patients with type 2 diabetes mellitus, keratinocyte-containing products should be used, since chronic hyperglycemia changes the morphology of cells, reduces cell proliferation and inhibits keratinocyte differentiation.

Available publications suggest that the use of dermatotropic BCPs with biodegradable collagen structures is promising. However, lack of comparative clinical studies and a single protocol can sometimes significantly reduce the importance of individual clinical observations.

It should be noted that when using dermatotropic BCPs, the wound itself must be adequately prepared. Preparation for application is a requirement for all BCPs before use to ensure the best possible outcome. Complex treatment, wound cleansing, including surgical treatment, reduction of infectious load, relief of the affected limb, daily care with assessment of the wound process dynamics can create prerequisites for successful use of BCPs in the treatment of chronic wounds.

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