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Chronic wounds and their treatment. Skin substitutes and allogeneic transplantations

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SUMMARY

A chronic wound is the loss of skin and other tissue, which does not heal in the period normally required for treatment of other wounds. Treatment of these wounds may be long and requires specific medical skills.
The causes of chronic wounds formation are variegated. The most frequently-observed are venous leg ulcerations, ischemic wounds, diabetic foot ulcerations and decubitus ulcerations. The CEAP classification system has been introduced in order to describe the condition of a person diagnosed with chronic venous insufficiency. Clinical, etiological, anatomical and pathophysiological aspects of the disease are monitored using this classification.
Surgical wound cleansing is the basis of all chronic wound treatment. In addition to typical surgical approach, there is a modern, promising methods, such as hydrosurgery and using insect larvae (eg. fly Lucilia sericata).
The possibility of replacing the damaged skin areas is significant for proper treatment. An important issue is the development of useful skin substitutes. Research are conducted in many research centers around the world. Medicine and biotechnology lead to the development of many useful skin substitutes, such as Biobrane, Integra, Dermagraft and other.
To conclude, the chronic wounds are a crucial problem from the medical viewpoint, but new and effective treatment methods are constantly being developed.
Key words: chronic wounds, classification, treatment, skin substitutes, transplantation

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CHRONIC WOUND – DEFINITION, CLASSIFICATION, CAUSES OF OCCURRENCE AND CLINICAL PICTURE

A chronic wound (CW) is the loss of skin and, alternatively, deeper layers of tissue, which, despite administering types of treatment similar to those applied in other cases, does not heal in the period normally required for treatment of other wounds. It is difficult to determine the exact time after which a wound can be considered chronic [1]. It is suggested, however, that a wound, which does not reveal signs of recovery after 21 days, be classified as chronic [2]. In the case of healing wounds, an equilibrium between production and degradation of such proteins as collagen is maintained; yet, in chronic wounds, the prevalence of degradation is symptomatic of this equilibrium having been upset.

Chronic wound treatment may be long, and take even years, and some of these wounds fail to heal permanently. For this reason, this process requires specific medical skills. Furthermore, patients with chronic wounds, frequently suffer both physically and mentally, as the non-healing wound becomes a source of stress, and, in many cases, even leads to depression [1].

Patients with chronic wounds frequently state, that pain is a dominant sensation in their lives [3, 4, 35]. Pain management is one of the most crucial aspects of treatment and cannot be omitted. Six out of nine patients who have developed venous ulcers, report to experience severe pain, and in the case of other chronic wounds, this proportion is similar. Furthermore, administering low-effective pain killers may even increase the patient's frustration.

The causes of developing chronic wounds are very variegated, and generally, possible to determine. The most frequently-observed are:

- venous leg ulcerations (ca. 75% of all chronic wounds),
- ischemic wounds (ca. 14% of chronic wounds),
- diabetic foot ulcerations (ca. 5% of chronic wounds),
- decubitus ulcerations (other) [1, 9].

Other injuries of mixed etiology, which are rarer to appear, involve: pyoderma gangrenosum, malignant cutaneous wounds, wounds occuring as a result of digestive system fistulas or sever injuries, as well as wounds of unknown origin [1].

Description of chronic wounds should start from venous leg ulcers due to their high frequency of appearance as well as the fact that they are considered to be the most severe form of circulatory failure [8].

Circulatory failure is defined as improper functioning of the cardiovascular system associated with insufficiency of vein valves, which are accompanied by the impaired venous blood outflow. This may concern the superficial or deep venous system, and may be inborn or acquired [5]. Despite the impairment of venous blood outflow being of utmost importance, the role of venous pressure, damages or pathological lesions in the veins themselves should not be disregarded. Therefore, chronic venous insufficiency is a condition in which the impaired venous blood outflow occurs on the permanent basis.

In normal conditions, deoxygenated venous blood flows away from the system to the heart, and from the superficial veins to deep ones. This process is maintained by the properly-working venous valves [6] as well as a powerful muscle pump. After a certain time, the improper functioning of these systems leads to the development of venous inefficiency. Among the symptoms of this disease one may find teleangiectasias (i.e. small dilated blood vessels), varicose veins, oedema, trophic changes, active and healed ulcerations.

In order to describe the condition of a person diagnosed with chronic venous insufficiency, the CEAP classification system has been introduced, allowing for monitoring various aspects of the disease (clinical, etiological, anatomical and pathophysiological) [7]. Various parameters may take values as mentioned below:

C (clinical aspect):
0 – no visible or palpable changes
1 – telangiectasies or reticular veins
2 – varicose veins
3 – oedema
4 – skin lesions (A: pigmentation, dermatitis, B: *lipodermatosclerosis*)
5 – healed ulceration
6 – active ulceration
A – asymptomatic

E (etiological aspect):
E_C – congenital syndromes
E_P – primary changes of unknown origin
E_S – secondary changes of known origin

A (anatomical aspect):
A_S – superficial veins:
1 – telangiectasies or reticular veins
2 – saphenous vein above the level of the knee
3 – saphenous vein below the level of the knee
4 – fibular vein
5 – other superficial veins
A_D – deep veins:
6 – inferior vena cava
7 – common iliac veins
8 – internal iliac vein
9 – external iliac vein
10 – pelvic veins
11 – femoral vein
12 – deep femoral vein
13 – superficial femoral vein
14 – popliteal vein
15 – deep crural veins
16 – musle veins
A_P – perforating veins (perforators):
17 – penetrating femoral veins
18 – penetrating crural veins

P (pathophysiological aspect):
P_R – reflux,
P_O – obstruction,
$P_{R,O}$ – reflux and obstruction [7].

The chronic wound development in the form of venous ulceration occurs as a result of necrosis caused by hypoxia. This is effected by the impaired venous blood outflow in the extremity. From this reason, venous pressure in lower extremities increases even in capillaries. This entails the opening of arteriovenous shunts and closing of pre-capillary sphincters; this disturbs microcirculation, and, as a result, leads to ischemia and necrosis.

Secondary etiology is usually based on deep vein thrombosis as a result of which venous blood enters the superficial venous system. This

causes reflux, which is later transferred through perforators to the superficial venous system and capillaries.

In order to explain the causes of the primary disturbance, two theories were formulated:

- hemodynamic (descending),
- impairement of the venous wall (ascending).

According to the first theory, an increase of venous pressure in the venous system, resulting from a variety of possible causes, is transferred from the right atrium to the veins of the lower extremities causing their dilation and reflux. The second theory says that the primary cause is the pathological condition of the wall of a venous vessel, which leads to its dilation (venous distention).

Another example of chronic wounds are ischaemic ulcerations, usually caused by atherosclerosis of the lower extremities. Rarer causes include circumferential aneurysms, acute ischemia, developmental disorders, states after injuries or surgical treatment, or inflammatory arterial diseases. Atherosklerosis consists in developing degenerative- productive changes in the internal and central membranes of the arteries. In the case of peripheral, as well as other types of vessels, an increasing stenosis of the arterial lumen occurs, leading to complete closure of arteries The result of this phenomenon is ischemia of the tissues supplied by damaged vessels, which causes their death and the emergence of necrotic tissue. The most frequently encountered disease (60% of cases) is femoral-popliteal occlusive disease. On the other hand, a total occlusion of arteries localized above the knee occurs the least frequently [9].

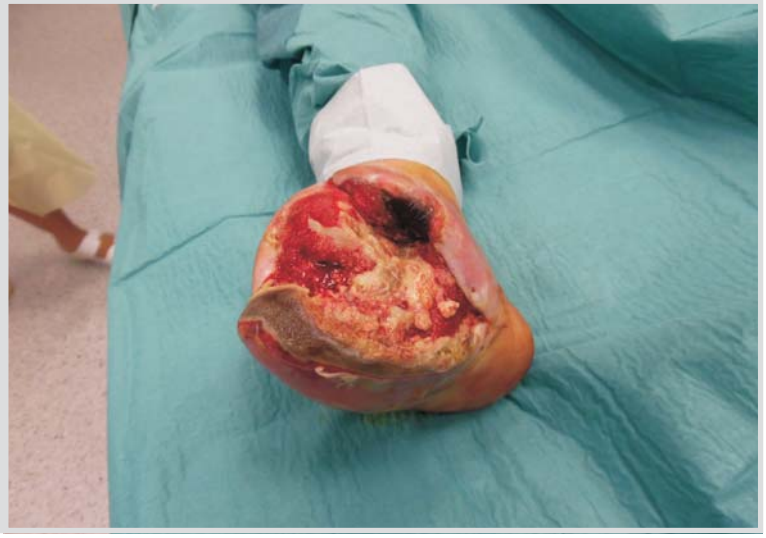
The causes of ulcerations caused by diabetes may be variegated. The most frequent are pathological changes which develop in the area of the lower extremities, particularly in the case of feet [Phot. 1]. Disorders associated with this disease include macroangiopathy microangiopathy and neuropathy.

Diabetic macroangiopathy is sometimes defined as the "premature atherosclerosis". Despite some similarities to atherosclerosis, in patients with macroangiopathy, some crucial differences occur in reference to the changes observed in those patients without diabetic disorders. In patients with diabetes diabetic disorders occur relatively early. The plaques are, in relation to typically atherosclerotic ones, more fragile – their cover breaks easily and this may lead to acute coronary syndromes or ischemic strokes. The vessels alone are characterized by high stiffness, which makes them less susceptible to hemodynamic changes [10].

Diabetic microangiopathy develops as an effect of the oxidoreductive imbalance of the system which results in generating higher-than-normal amount of free radicals; these influence the increase of oxidized LDL cholesterol concentrations that possess cytotoxic properties. Hyperglycemia leads to the increase of the non-enzymatic glycosylation of proteins. Hyperglycemia is also the cause of increased production of triglycerides. This leads to endothelial dysfunction [11].

Diabetic neuropathy is a damage of sensory nerve fibers in the tissue of the foot which may, inter alia, lead to the problems in blood-flow regulation.

Phot. 1. Complicated case of diabetic foot



Due to heterogeneous causes of chronic wounds, their clinical picture is much variegated. The existence of a chronic wound is associated with constant presence or re-emergence of a factor inducing it. Therefore, a healing process is simultaneous with a pathological process.

Despite direct factors, which may inhibit wound healing, there are also general factors that may prolong the healing process. Among them, one can distinguish:

- malnutrition, general weakness,
- circulatory and metabolic diseases,
- neoplasms,
- chronic infections,
- obesity,
- vitamin C deficiency [13],
- old age.

Undoubtedly, this is not a complete list of causes which influence chronic wound development. There are also local factors, such as:

- the presence of necross,
- bacteria colonizing the wound,
- excudate,
- interstitial oedema,
- chronic injuries.

Venous leg ulcerations occur most frequently in the lower extremity, in the areas below the knee. The changes usually occur above an ankle, and their localization may vary during the development of the disease. Ischaemic ulcerations occur most frequently in the lower extremity, but usually above bony prominences, e.g. on the dorsal side of toes. Ischemic venous ulcerations display more complex symptoms, and their picture is atypical. Diabetic complications may bear results similar to ulcerations caused by atherosclerosis, including necrosis [14]. When not treated properly, all changes reveal a tendency to expand.

A decubitus ulcer is a locally-limited necrotic tissue. This condition may concern epidermis, the overall skin thickness, subcutaneous tissue, muscles and bones. Decubitus ulcer develop as a result of the prolonged or repeated pressure, which causes hypoxia, and subsequently, their death. In chronically-ill patients, ulcer appear in areas, which touch the surface, mainly in the area of the coccyx, the sacrum, buttocks (Phot. 2), on soles or hips [12]. The pressure may be caused by:

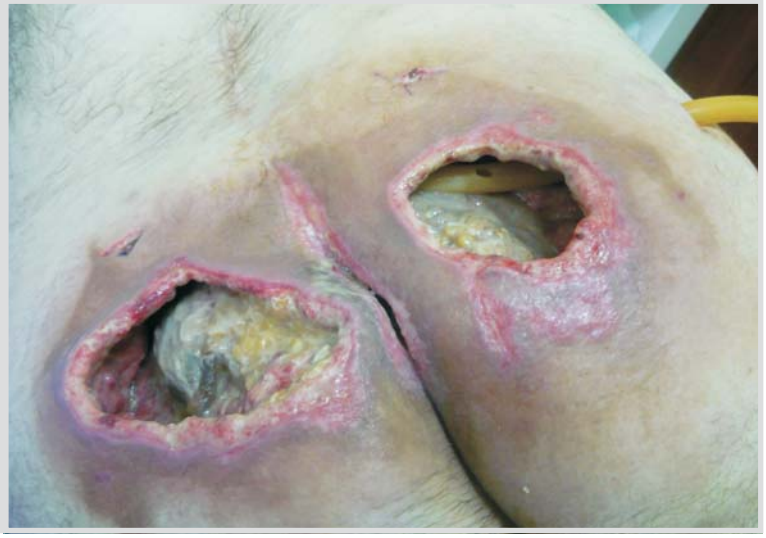
- direct pressure applied to the soft tissues by the bone structure on one side, and hard surface on the other,
- the friction of skin against another surface which leads to the loss of epidermis,
- by shear forces appearing as a result of moving a patient on the surface [9].

Decubitus ulcers occur in lying patients, or those with limited movability. A high risk of developing pressure wounds appears in people with obesity where the force of pressure, associated with gravity, is of higher value.

Some areas of the body are predisposed to develop pressure wounds. These are areas where bony prominences are localized rather closely to the soft tissue, inducing pressure on tissues lying above them, normal blood flow through the veins.

Clinical practice deemed it necessary to formulate and implement a uniform way of describing the condition of chronically-ill pa-

Phot. 2. Massive decubitus ulceration (male buttocks)



tients with decubitus ulcers. For this purpose, in 1983, the Torrence system was defined, presenting five stages ranked in accordance with the increase of danger [15]:

- Blanchable erythema (stage I) – visible hyperaemia and reddening of the skin. Local microcirculation is not yet physically damaged, and localized delicate pressure results in its disappearance.
- Non-blanchable erythema (stage II) – visible hyperaemia and reddening of the skin. However, contrary to stage I, the change persists even after removing pressure. The patient usually experiences pain, caused by oedema and advancing damage to the circulatory system. Epidermis is susceptible to damages, and blisters.
- Full thickness damage (stage III) – sharp separation of the the wound margin from adjacent tissue. The tissue is surrounded by oedema and erythema. The wound is filled with granulation and necrotic debris.
- Subcatenous skin damage (stage IV) – adipose tissue necrosis caused by capillaries damage. The wound bed may be lined with eschar. Frequently, the pressure wound has well-defined margins, but sub-surface necrotic expansion may ocur.
- Advanced necrosis (stage V) – joints and bones may be fully damaged or destroyed [15].

Another cause of chronic wounds may be cancerous diseases, e.g. Marjolin's ulcers. This neoplasm develops as a result of chronic ulcerations or scars, and is a type of basal cell carcinoma. Chronic tissue irritation is a vital factor, as neoplasm develops in this area. Therefore, this type of cancer occurs in post-surgical stumps, and also as a result of using prosthesis [16][17]

CHRONIC WOUNDS TREATMENT

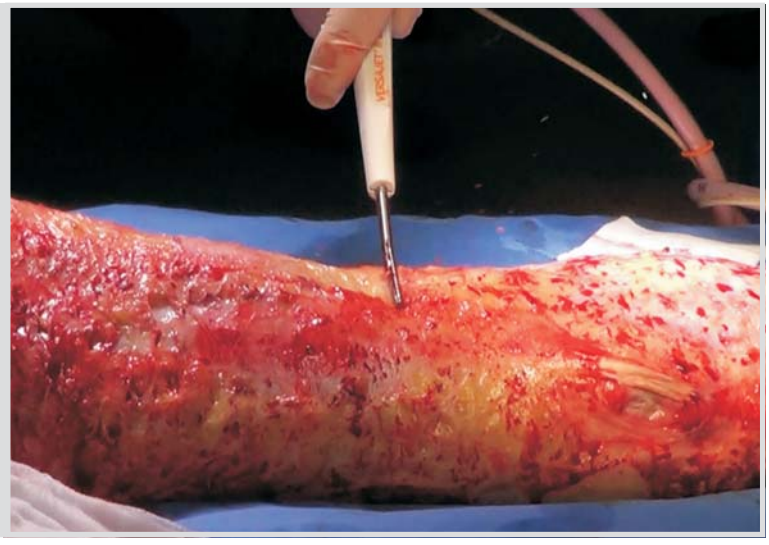
The basis of all chronic wound treatment is a surgical wound cleansing. During this procedure, all infectious factors and decaying necrotic tissue are removed, which commences an effective process of healing. Damaged tissues are removed together with a margin of healthy tissue in order to prevent infectious and necrotic expansion.

Hydrosurgery is a a state-of-the-art surgical technique, which involves wound cleansing by means of a high-pressure stream of physiological saline. The tip of the cutting tool moves along the surface of the wound, and a thin stream eliminates necrotic tissue and bacteria (Phot. 3). At the same time, this technique causes little damage to healthy tissue. This procedure enables for a quick and precise chronic wound cleansing [2].

The wound cleansing process is supported by a locally-induced negative pressure therapy (MTP, eng. *topical negative pressure therapy* TNPT), mobilizing the body to regenerate [36] (Phot. 4).

Other means of wound cleansing involve using insect larvae. The most commonly used are the larvae of a *Phaenicia sericata* fly (also know under the name *Lucilla sericata*) of the *Calliphoridae* family, which are cultivated in special conditions, so that they were not hosts for micro-organisms and viruses.

Phot. 3. Hydrosurgery wound cleansing (burn case)



The advantage of fly larvae is that they feed only on tissue damaged by disease, e.g. necrotic tissue, with no harm to the healthy tissue [18]. In many places of the world, cleansing wounds using larvae is used in the case of pressure wounds, venous ulcerations, diabetic foot and other chronic wounds, in particularly those localized on the limbs (Phot. 5). Yet, this method is still a source of controversion – the probable cause being an instinctive aversion to fly larvae, which are considered dirty and likely to spread diseases. These claims are unfounded as the larvae are sterilized by means gamma radiation.

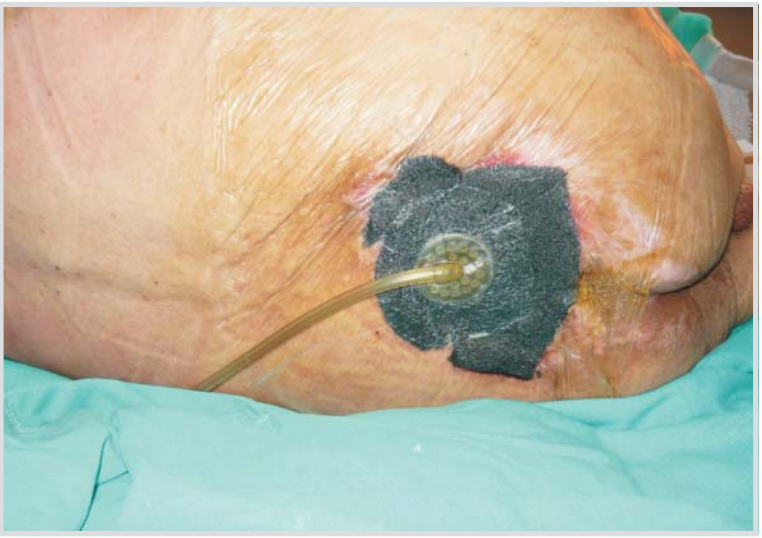
Despite wound cleansing, the introduction of treatment and healing, it is frequently necessary to compensate for the tissue loss including skin loss.

The skin, as the largest human organ, possesses a complex structure and performs numerous functions such as:

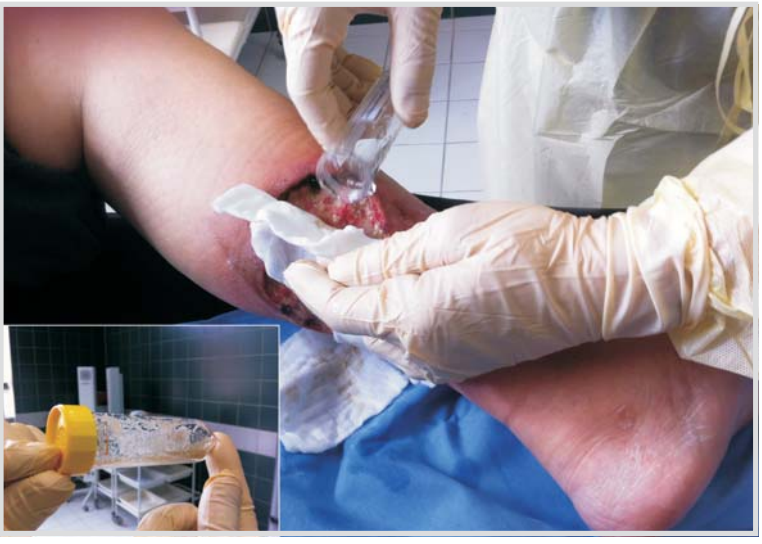
- isolating the body from the external environment; in other words, participation in maintaining homeostasis [19, 20],
- ensuring thermoregulation,
- owing to melanine present in melanocytes, it protects the body against UV radiation,
- enabling the perception of stimuli from the external environment, e.g. temperature, touch,
- enabling the production of vitamin D [21],
- helping the endocrine system,
- and many others.

In patients with chronic wounds, large skin areas may be damaged. As the skin constitutes a protective barrier against environmental fac-

Phot. 4. Case of decubitus ulceration treated with TNPT system



Phot. 5. Application of Phaenicia sericata larvae in case of trophic ulceration; Left-bottom – container with larvae



tors, its poor condition is highly damaging due to infections alone, and may prevent or hinder further treatment. The aesthetic aspect should not be omitted: in future, large skin loss may result in visible, disfiguring scars.

The possibility of replacing the damaged skin areas, that is compensating for skin loss in the case of chronic wounds, is also a vital aspect of treatment. The first, at least partially successful skin transplants, were conducted ca. 1870 by Riverdene [22].

Since these pioneering actions, skin transplantations, coming both, from the same patient (autologous), as well as other donors (allogeneic), have become generally performed surgical procedures [23]. They possess numerous advantages – from the clinical viewpoint, the most important one is that skin transplantaions are relatively easy to perform, and while using perfected techniques, they do not bear (in comparison to other transplantations) a risk of complications. The procedures also have certain disadvantages. In the case of massive burn and large chronic wounds, it may be difficult or impossible to collect a proper amount of skin graft for the transplantation. Another problem is finding a different donor for this procedure [24].

For these and other reasons, the works on developing useful skin substitutes are constantly being conducted. The basis for the research was created by an English surgeon, Joseph Samson Gamgee, who, in 1880, created a cotton wool dressing, placed between gauze [25]. Further research revealed that epidermic tissue, as well as the skin, may survive outside the body [26], and subsequently, be transplanted as proved by Lunggren's transplants. This breakthrough did not occur until 1975, when Rheinwald i Green successfully cultivated human keratinocytes on the basis of fibroblast cells derived from mice [27].

Today medicine and biotechnology lead to the development and implementation of many skin substitutes, both those that contain living cells taken from the patient's body, as well as those devoid of them.

The non-cellular substitutes include Biobrane, Integra or Alloderm.

Biobrane is composed of the underside, nylon, openwork mesh and a silicone membrane on the surface. Porcine collagen is present, in both structures [27]. This substitute is frequently used as a temporary wound dressing.

Integra in the most well-known product which replaces skin during the post-burn treatment. It is a two-layer structure, and its outer layer has a form of a silicone membrane which is impervious to water, and protects against infections. Its underside layer is made of bovine collagen with an addition of chondroitin sulphate,and glycosaminoglycan from a shark [28]. After covering the tissues with Integra,the tissues undergo revascularization within a few weeks. After that time, the surface layer is replaced by a very thin skin graft. The substitute gives good effects, even on a large wound surface, but its major disadvantage is its high price and the necessity of conducting at least two surgeries.

Alloderm has a completely different structure: it is a non-cellular matrix, obtained from dead dermis. Firstly, dermis is treated with sodium chloride in order to remove epidermis, and then, it is repeatedly washed in its solution to remove all tissues. The remaining part is then freeze-dried so as not to evoke the immunological response [29].

When it comes to cellular skin substrates, one needs to specify, as in the case of transplantations, the allogeneic and autologous substitutes. Tissue cells, and keratinocytes in particular, may be collected from living donors, and then cultivated via *in vitro*. Specific cell cultures generated by this method are treated as transplants which support a therapy of slow-healing wounds [30].

Allogeneic substitutes contain living cells in their structure, which are taken from related or unrelated donors. Dermagraft i Apligraf are the most frequently used skin subtitutes [23, 37].

Dermagraft is composed of a mesh generated in process of mixing living fibroblasts, which are taken from the infant's foreskin, with the medium in the form of a PGA-construted mesh, fully biodegredable in the human body. Fibroblasts are cryopreserved at a temperature of -80°C so that they remained alive until their final transplantation into the wound. When in the wound, they start to proliferate and generate healing-inducing factors. The poliglycol mesh is absorbed fully within ca. one month.

Dermagraft is useful in chronic wounds treatment of diabetic foot or venous ulcerations. This substitute provides the body with rich-in-collagen matrix that contains metabolically active fibroblasts. Dermagraft alone tentatively replaces skin, but simultaneously stimulates the wound's healing process, which enables even slow-healing wounds, or chronic injuries to heal [31, 32, 33].

Apligraf is the most advanced skin substitute. It was introduced to the clinical use in 1998.

The production process of this artificial tissue is relatively complex, even when compared to other substitutes. At the tentative stage, fibroblasts taken from infant's foreskin are mixed with Type I bovine collagen, but the mixture is exposed to a high temperature in order to create a loose matrix. The material is left for the period of two weeks during which new collagen is generated and a thick mesh of fibers is created within the matrix. Such a generated matrix is seeded with the suspension containing living cells (taken from the same or another infant) and then left for 4 more days to induce proliferation. In the period of the last two days, the calcium ion concentration increases in the cell culture, which results in generating a new layer of the stratum corneum. At this stage, the substitute is ready.

The application of Apligraf produces wonderful results in treating venous ulceration and the cases of diabetic foot that are resistant to other forms of treatment. Research revealed that a therapy utilizing skin substitutes is more effective than other therapies in relations 69%:49% in patients who experienced a full recovery within 6 months [34].

Autologous skin-replacement materials are generated in the process of isolating cells taken from the patient, and their subsequent cultivation and transplantation.

To conclude, the chronic wounds constitute a vital problem from the medical viewpoint. They are difficult to treat, and a therapy is costly, both in terms of time and finances dedicated to treatment. Transplantation and substitutes are the solution that enables recovery in a much shorter period than in the case of utilizing other techniques. Application of autogenous transplantations is particulatly advantageous. One should not underestimate the value of transplants taken from other donors, as they still constitute a significant percentage of all transplantations.

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