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# BIOTECHNOLOGICAL METHODS OF LOCAL TREATMENT OF INFECTED WOUNDS IN DIABETES MELLITUS IN AN EXPERIMENT

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**Abstract**

Experimental type I diabetes mellitus was replicated on 54 white adults Wistar rats of both sexes, weighing 220 ± 12.56 grams, by administering Alloxan (15 mg/kg) into the tail vein. Infected wound in a square-shaped interscapular area with a total area of 400 $mm^2$ was simulated on 48 animals and divided into two groups: control and experimental. 6 animals were included in the intact group, where the indicators were considered normal. It was found that under the action of allofibroblasts there is an early cleansing of the wound from the remnants of necrotic tissue and a faster rate of epithelialization. Due to the significant role of lipid peroxidation processes in the course of inflammatory reactions, a positive effect was found in the study group on TBA-active products and indicators of antioxidant enzyme activity. The dynamics of non-specific immunity, the cellular part of the immune system, as well as the content of circulating immune complexes indicates a positive effect of this dressing on the wound process as a whole, due to the presence of growth factors as equivalent to the dermismanuscript.

**Keywords**: *type 1 diabetes mellitus; infected wound, antioxidants, growth factors, regeneration*

## Introduction

Patients with infected soft tissue defects of the lower extremities make up a significant proportion of the total number of surgical patients. Among the scientific works on the study of the processes that occur in wound defects at the cellular-molecular level and prevent their healing, of particular interest are the works on the pathologies of diabetes. Thus, it is known that the contraction of the wound defect and the formation of granulation tissue are mainly due to the activity of fibroblasts [1, 2, 3], which affect wound healing and epithelialization due to their ability to produce collagen of different types [1, 2] and growth factors [1, 4, 5]. In addition, it was found that in wounds that do not heal for a long time on the background of diabetes, the proliferative and metabolic activity of fibroblasts is significantly reduced [1, 6]. As a result of pathological modification of the receptor apparatus, functioning cells become insensitive to the influence of certain growth factors [7, 8]. Therefore, the use of active growth factors is favorable for the restoration of epithelialization processes. From this position, it is interesting to develop new technologies for the regulation of the wound process in the complex surgical treatment of patients with wound defects in diabetic foot syndrome [5, 9, 10].

Despite the extensive experience and ongoing research on the development of modern methods of treatment, wound healing remains one of the pressing problems of surgery [9, 11]. The results of observations show that modern drugs when used gradually lose their effectiveness [6, 9, 10, 12, 13].

It is known that repair processes take place under the control of growth factors, which are present in the body in small quantities but have a significant impact on wound healing. Many cells need a small amount (approximately $10^{10}$ mol/L) of growth factors to survive and proliferate [6, 14]. Among the currently known polypeptide growth stimulants are fibroblast growth factors. The latter plays an important role in many biological functions, including the development and maturation of structures, cell differentiation, and regulation of metabolism, tissue repair, angiogenesis, and mitogenesis [15]. Fibroblasts are also able to synthesize tropocollagen, collagen precursor, intercellular matrix, and the main substance of connective tissue, an amorphous jelly-like substance that fills the space between cells and connective tissue fibers. These cells are actively involved in the healing process of wounds [7, 16]. Of interest for the experimental study will be the local application of the dermis equivalent (ED) based on skin allofibroblasts on the simulated infected wound process.

**The aim of the study** was to determine the effectiveness of allofibroblasts on the healing of infected dermatome wounds in experimental animals.

## Methods

The work was performed on 54 white adult Wistar rats of both sexes with an initial weight of 220 ± 12.56 g, type I diabetes mellitus was simulated by injection of Alloxan into the tail vein (15 mg/kg). The development of insulin insufficiency was confirmed after 14 days by assessing glycemia (more than 12 mmol/L). All animals were kept on a standard I.Horbachevsky Ternopil National Medical University vivarium diet, kept under standard conditions at room temperature in isolated cages with a 12-hour day/night, with access to water and food ad libitum. The studies were carried out in accordance with national and international recommendations for the protection of animals used for experimental and other scientific purposes (Strasbourg, 1986; Law of Ukraine № 3447-IV, 2006).

The regenerative potential of ED was studied on a model of a standard infected dermatome wound, modeled in the interscapular area of experimental animals using a prefabricated square stencil with a total area of 400 $mm^2$. Tissues were excised together with the superficial fascia. To reproduce the wound infection used a strain of pathogenic *S. Aureus*, which was grown on a nutrient medium. The edges of the wound were fixed to the deeper soft tissues with simple nodal sutures, due to the fact that in experimental animals for 2-3 days after wound formation, there is a significant contraction of the wound, which leads to a rapid decrease in the wound areas and inability to assess the effect of local treatment on the epithelialization of the defect. Therefore, we thus avoided wound healing by contraction and were

able to more accurately assess wound healing by affecting the rate of epithelialization.

The ED is a three-dimensional structure consisting of human allofibroblasts enclosed in a collagen gel (Fig. 1). Isolation, cultivation and seeding of allofibroblasts were performed in the laboratory. Cells of 4-6 passages were used to create ED. Collagen gel was prepared according to standard methods from a solution of type I collagen obtained by extraction with acetic acid. ED was prepared in plastic Petri dishes by mixing a solution of collagen and a suspension of fetal fibroblasts by the method of E. Well et al.

The first – intact group includes animals that were not simulated the wound process and did not receive treatment (6 animals). Animals of the second group – control group, simulated the wound process and did not use any method of influencing the wound process, the wound on the back was open all the time, healed on its own by secondary tension (24 animals). Animals of the third group, starting from the day after the reconstruction of the wound, once-daily applied the equivalent of the dermis on the basis of allofibroblasts (24 animals). The healing properties of ED were studied on days 3, 5, 7, 10, 14, and 21. Euthanasia was performed by anesthetic overdose on the 3rd, 7th, 14th and 21st days. In the soft tissues of the wound were determined by the content of lipid hydroperoxides (LHOP), TBA-active products (TBA-AP), the activity of superoxide dismutase (SOD) and catalase (CAT). In the peripheral blood was determined by the phagocytic activity of leukocytes (PhAL), the content of T- and B-lymphocytes. In blood serum - the bactericidal activity of blood serum (BABS), lysozyme activity of blood serum (LABS), the complementary activity of blood serum (CABS), level of the general circulating immune complexes (CIC).

## Results and Discussion

According to the terms, the wounds were photographed and the edges of the wound were copied on transparent paper, in order to determine the dynamics of its change in each group of animals, followed by determining the rate of epithelialization. Observations showed that in each group of animals on the third day the processes of a post-traumatic inflammatory process dominated, the edges of the wound were roller-like, swollen, the wound was covered in places with thick brown crusts, and the bottom was hyperemic. These signs were most pronounced in animals of the control group.

Subsequent changes in the condition of wounds and the course of healing were registered in the subsequent terms of observation. On the 5th day in the control group, there were further signs of inflammation, edges were swollen, roller-like, the wound was covered with a layer of crusts. The condition of the animals included in the experiment differed significantly less hyperemia and edema; the wounds were covered with a thin layer of fibrous film. At this time, the experimental group showed no signs of inflammation, the wound was clean, covered with a thin layer of fibrous film, and visually the wound was clean.

On the 7th day, the area of the wound was significantly reduced in animals to which allofibroblast-based dressings were applied, the edges of the wound were close to its bottom, where the appearance of islands of granulation tissue was visually observed.

The subsequent stages of wound healing in all groups were characterized by the development of granulation tissue, which was covered with epithelium from the edges. A much stronger dominance of these processes was in the experimental group (+ED).

On the 14th day, the condition of the wounds was characterized by a further process of epithelialization from its edges and, accordingly, a decrease in the area of the wound. Significant positive changes in the animals of the experimental group treated with ED were that they had a small pure oval-stretched wound in the middle of the area where the primary wound was modeled. On the 14th day, the wounds in the animals of the control group were larger.

At the end of the observation, on the 21st day, the wound healed almost completely in the group of animals treated with ED, there was only a small area covered with a dense scab. The reduction in the size of the wound and the deadlines for healing indicated the speed of regenerative processes. The data obtained are shown in table 1.

The measurement data correlate with the clinical and visual stages of wound healing. In all

terms of the study, there were positive dynamics of wound healing in both groups.

Due to the significant role of lipid peroxidation processes in the course of inflammatory reactions, including those caused by the wound process, we studied the intensity of accumulation of intermediate (LHOP) and end (TBA-AP) lipoperoxidation products and the state of the enzyme link of the antioxidant system (SOD and CAT) in the soft tissues of the wound [17]. The results are presented in table 2.

From the above results, it is seen that the infected wound significantly increases the intensity of production of intermediate products of lipoperoxidation – lipid hydro peroxides. In particular, on the 3rd day from the moment of inflicting the wound, the indicator in the group where no effect on the wound process was used exceeded the level of intact animals 1.6 times and continued to grow until the 21st day, when it was 242% of normal. This indicates the activation of oxygen-dependent processes in tissues caused by the mobilization of peripheral blood mononuclear phagocytes and their generation of reactive oxygen species in order to liquidate the infectious agent.

TBA-active products also grew significantly, even more intensively than LHOP. Thus, on the 3rd day the indicator was 168% of the intact group, on the 7th – 218%, on the 14th – 647%, and on the 21st – 282%.

One of the reasons for this growth, along with the intensive formation of reactive oxygen species, may also be a decrease in the rate of their inactivation by components of the antioxidant system. In the experimental group with the use of ED, wavy dynamics of the activity of key enzymes of antioxidant protection – SOD and CAT – were observed. In particular, the activity of SOD on the 3rd day was 134% of intact animals, but in subsequent follow-up periods decreased significantly and amounted to 78, 76 and 81%, respectively. Similar changes were observed for catalase - an increase of 1.3 times on the 3rd day and a decrease in subsequent follow-up, and on the 21st day was 91% of normal.

The use of allofibroblast dressings had a slightly different effect on the parameters studied. In particular, the concentration of LHOP in comparison with the animals of the control group was higher on the 3rd day of the study and amounted to 105% of their level. Subsequently, the indicators continued to decrease and by the 21st day, the LHOP content was significantly different from the same indicator of the control group. A more positive effect of ED was found for TBA-active products. On the 3rd day of observation, the indicator did not differ significantly from the level of the control group, but by the 21st day, it was still 2 times smaller in the experimental group (+ ED).

The activity of antioxidant enzymes under the action of ED increased. If in the initial observation period this increase was not significant, then by the 14th and 21st days of the studied indicators were significantly higher than the level of the control group.

The positive effect of ED is also indicated by the dynamics of non-specific immunity (table 3) [18, 19]. If in the late stages of the infected wound process, we recorded a significant decrease (after a short increase on the 3rd day) phagocytic activity of leukocytes, the bactericidal activity of blood serum, lysozyme activity of serum, the complementary activity of serum, which indicates depletion of immune reserves group without the use of any treatment. In the group with the use of ED, these indicators were significantly restored and by the 21st day approached the level of the intact group of animals.

Another confirmation of the positive effect is the positive dynamics of the cellular component of the immune system, as well as the content of circulating immune complexes (Table 4). In the group using ED recorded complete normalization of B- and T-active lymphocytes, as well as a decrease in the content of circulating immune complexes in the serum by 17.3% compared with the control group of animals on the 21st day, which indicates a more favorable course wound process in the group using ED.

## Conclusions

The analyzed data indicate that the rapid healing of experimental wounds with the use of bandages based on allofibroblasts is due to the intense impact on the initial phases of regeneration, while reducing the manifestation of post-traumatic inflammatory process. At 5-7 days was the greatest rate of wound healing. This is due to the inhibition of

microorganisms in the wound, which contributes to the interruption of the inflammatory chain, which helps to clean the wound and the development of granulation tissue.

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**Figure 1.** The structure of the equivalent of the dermis

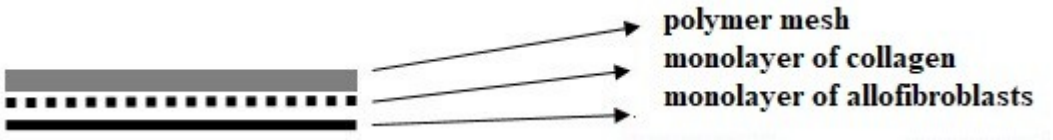


**Table 1.** Change in the area of infected dermatome wounds (M ± m)

| The day of the study | Wound area, $mm^2$ | |
|---|---|---|
| | ***Control group* (was not treat)** | ***Experimental group* (+ ED)** |
| Initial data | 400 | 400 |
| 3rd | 397±22.4 | 381±15.8* |
| 5-th | 384±18.5 | 324±14.7*/** |
| 7-th | 371±16.7 | 270±14.2*/** |
| 10-th | 314±13.3 | 187±12.4*/** |
| 14-th | 232±11.5 | 128±8.9* |
| 21-st | 84±8.7 | 31±5.1* |

* - the difference is significant compared to animals that were not treated

**Table 2.** Indicators of lipid peroxidation processes and the state of the antioxidant system in the soft tissues of animals in dynamics (M ± m)

| Groups of animals | | | | |
|---|---|---|---|---|
| ***Intact group*** | ***Control group* (was not treat)** | | | |
| | 3rd day | 7th day | 14th day | 21st day |
| LHOP, $*10^3$, standard unit/L | | | | |
| 1.41±0.01 | 2.30±0.02* | 2.52±0.02* | 3.20±0.03* | 3.41±0.03* |
| TBA-AP, nmol/g | | | | |
| 1.02±0.02 | 1.71±0.02* | 2.23±0.02* | 6.60±0.02* | 2.92±0.03* |
| SOD, standard unit/mg protein | | | | |
| 29.3±1.2 | 37.4±2.1* | 19.1±1.7* | 14.5±1.4* | 14.0±1.4* |
| Catalase, kmol/(min*mg protein) | | | | |
| 39.3±1.2 | 48.4±1.8* | 25.6±1.8* | 21.3±1.7* | 20.8±1.7* |
| | ***Experimental group (+ ED)*** | | | |
| | 3rd day | 7th day | 14th day | 21st day |
| LHOP, $*10^3$, standard unit/L | | | | |
| 1.41±0.01 | 1.73±0.02** | 1.90±0.02** | 2.36±0.03** | 2.53±0.03** |
| TBA-AP, nmol/g | | | | |
| 1.02±0.02 | 1.27±0.02 | 1.24±0.02 | 3.31±0.02 | 1.42±0.03 |
| SOD, standard unit/mg protein | | | | |
| 29.3±0.2 | 39.4±2.4 | 23.1±1.5** | 22.5±1.2** | 24.0±1.2** |
| Catalase, kmol/(min*mg protein) | | | | |
| 39.3±0.2 | 53.4±1.4** | 34.6±1.6** | 30.3±1.5** | 35.8±1.4** |

Notes: * - the difference is significant relative to the intact group

** - the difference is significant relative to the control group

**Table 3.** Indicators of nonspecific immune protection of animals in dynamics (M ± m)

| Groups of animals | | | | |
|---|---|---|---|---|
| ***Intact group*** | ***Control group* (was not treat)** | | | |
| | 3rd day | 7th day | 14th day | 21st day |
| PhAL, % | | | | |
| 55.6±1.3 | 62.7±1.4* | 67.5±1.5* | 43.4±1.2* | 42.1±1.2* |
| BABS, % | | | | |
| 25.8±1.5 | 33.9±2.1* | 37.6±2.4* | 19.4±1.8* | 19.1±1.8* |
| LABS, % | | | | |
| 40.6±0.7 | 45.3±2.2* | 46.4±1.9* | 34.2±2.1* | 33.8±2.0* |
| CABS, standard unit | | | | |
| 0.031±0.005 | 0.034±0.005 | 0.056±0.007* | 0.039±0.005* | 0.021±0.004* |
| | ***Experimental group (+ ED)*** | | | |
| | 3rd day | 7th day | 14th day | 21st day |
| PhAL, % | | | | |
| 55.6±1.3 | 68.3±1.1** | 69.4±1.7 | 61.4±1.3** | 53.1±1.1** |
| BABS, % | | | | |
| 25.8±1.5 | 38.2±1.3** | 37.1±1.6 | 29.5±1.4** | 26.2±1.2** |
| LABS, % | | | | |
| 40.6±0.7 | 48.5±1.4 | 47.3±1.7 | 39.5±1.4** | 41.2±1.3** |
| CABS, standard unit | | | | |
| 0.031±0.005 | 0.039±0.004 | 0.055±0.005 | 0.041±0.004 | 0.034±0.004** |

Notes: * - the difference is significant relative to the intact group

** - the difference is significant relative to the control group

**Table 4.** Indicators of cellular immune defense of animals in dynamics (M ± m)

| Groups of animals | | | | |
|---|---|---|---|---|
| ***Intact group*** | ***Control group* (was not treat)** | | | |
| | 3rd day | 7th day | 14th day | 21st day |
| B-lymphocytes, % | | | | |
| 44.3±1.4 | 48.9±1.0* | 51.1±1.2* | 43.8±1.4 | 44.9±1.4 |
| T- lymphocytes, % | | | | |
| 18.2±0.8 | 18.4±0.8 | 13.6±0.9* | 12.4±0.6* | 12.1±0.6* |
| CIC, mmol/L | | | | |
| 65.3±1.9 | 79.8±2.6* | 86.3±2.8* | 97.4±3.8* | 98.5±3.9* |
| ***Experimental group (+ ED)*** | | | | |
| | 3rd day | 7th day | 14th day | 21st day |
| B-lymphocytes, % | | | | |
| 44.3±1.4 | 45.6±1.1 | 47.3±1.1** | 44.2±1.2 | 44.7±1.1 |
| T- lymphocytes, % | | | | |
| 18.2±0.8 | 19.8±0.8 | 15.7±0.7 | 17.5±0.5** | 18.7±0.4** |
| CIC, mmol/L | | | | |
| 65.3±1.9 | 71.2±2.1** | 73.4±2.5** | 76.8±2.6** | 81.2±2.3** |