The tricky of healing persistent skin defects complicated by venous insufficiency, remains actual despite a large number of known methods of treatment, application of various dressings, and dynamically developing methods of tissue therapy [1]. Fibroblasts, mainly composed of collagen fibers and having the property to synthesize actively intercellular substances, are known to be the essential cells efficient in the formation of granulated tissue with subsequent ulcer cicatrization [2]. It is a known fact that fibroblasts and collagen fibers actively react and work with each other [3]. Fibroblasts can slide along self-synthesized collagen fibers, which makes them an excellent matrix or scaffold for tissue engineering construction [4]. Collagen is produced by extraction from the connective tissue of mammals (rats or


The problem of healing long-term skin defects, complicated by venous insufficiency, remains a problem despite many known treatments. The article researches immunological compatibility and efficiency of the use of collagen sponges with skin allogeneic fibroblasts for stimulation of wound healing. Sixteen mature C57/B1 mice were divided into two equal groups: control and experimental. In the 10th-day dynamic, the stimulation method was found to be highly effective by associating the collagen sponge with the allogenfibroblasts, which allows a significant improvement in the morphological characteristics of the sample biopsy and does not cause an autoimmune response to the transplant.

Keywords: ischemic cutaneous wound, collagen sponge, fibroblasts, T-lymphocytes

Проблема заживления длительно существующих дефектов кожных покровов, осложненных венозной недостаточностью, не теряет своей актуальности, несмотря на массу предложенных способов лечения. Было проведено исследование иммунологической совместимости и эффективности применения кollагеновой губки с дермальными аллогенными фибробластами для стимуляции ранозаживления. Шестнадцать половозрелых мышей линии C57/B1 были разделены на две равноценные группы: контроля и экспериментальную. В динамике к 10-м суткам выявлена высокая эффективность метода стимуляции ассоциацией кollагеновой губки с аллофибробластами, позволяющего существенно улучшить морфологические характеристики биоптатов и не вызывающего аутоиммунной реакции на трансплант.

Ключевые слова: ишемизированная рана кожи, кollагеновая губка, фибробласты, T-лимфоциты


The tricky of healing persistent skin defects complicated by venous insufficiency, remains actual despite a large number of known methods of treatment, application of various dressings, and dynamically developing methods of tissue therapy [1]. Fibroblasts, mainly composed of collagen fibers and having the property to synthesize actively intercellular substances, are known to be the essential cells efficient in the formation of granulated tissue with subsequent ulcer cicatrization [2]. It is a known fact that fibroblasts and collagen fibers actively react and work with each other [3]. Fibroblasts can slide along self-synthesized collagen fibers, which makes them an excellent matrix or scaffold for tissue engineering construction [4]. Collagen is produced by extraction from the connective tissue of mammals (rats or
A collagen sponge results from restoring collagen fibers using a solution [8]. The collagen sponge produced by the pharmaceutical industry is an excellent matrix for linking with skin fibroblasts [9] and subsequent transplantation into a persistent ulcer, even complicated by exudation. However, there is no record of tissue regeneration, including neoangiogenesis, in the second stage of the wound process.

The purpose of the study was the morphological analysis of tissue regeneration and angiogenesis in the presence of T-lymphocytes on the 10th day of ischemic skin healing of wounds after the transplantation of a collagen sponge with allogeneic dermal fibroblasts.

Material and Methods. The researchers used 4–6 month laboratory white mice of line CS7/81 divided into experimental and control groups, each containing 8 mice. Simulation persistent ischemic cutaneous wound technique was described in Baranovsky Yu. G. et al., 2016 [10]. Dermal fibroblast production and growing technique by an enzymatic method are present in the article [11]. A collagen biodegradable sponge was manufactured by LLC «Luzhsky Zavod Belkozin» (Luga, Leningrad region, Russia) using collagen solution. It contains boric acid and furacillin. A sponge is made as a dry yellow porous membrane possessing the power of high adhesion to the wound surface. Being dropped in the wound, the sponge gradually undergoes degradation [12]. The sponge with 1–1.5 million dermal all fibroblasts of 2–3 passage in alpha-MEM (Lonz) growth medium was transplanted into a simulation wound in mice. The wound was covered with Voskopran (Biotekpharm, Russia) sterile dressing and sutured to a silicone ring to hold the wound edges [13].

The healing sample from the wound biopsy was removed on the 10th day after the surgery. For morphometric and immunohistochemistry studies, we have prepared paraffin sections of the biptic and have tended to stain them with hematoxylin and eosin in a proper sequence. We measured the thickness of the epidermis, collagen fibers, and microcirculation vessels area using Image J (NIH Image, USA) under a total magnification of 400, 50 measurements per 1 section. According to an immunohistochemical method, T-lymphocytes were counterstained in paraffin sections with diaminobenzidine. CD3 (SP7) primary antibodies (ab16669) (Abcam, UK) at the ratio of 1:100 were used. T-lymphocyte number was determined by counting the amount of CD3-positive cells per 100 cells with subsequent ratings on a percentage base.

The obtained data were processed with the package STATISTICA 10.0 (StatSoft Inc., USA). We used the Mann – Whitney test with a p=0.05 significance level. The comparison of T-lymphocyte number, average thickness of the epidermis, collagen fibers, and microcirculation vessels area in the granulation tissue in experimental biopsies was carried out on a percentage base against the control group.

Results and Discussion. On day 10, after modeling a persistent ischemic skin wound and closing it with a collagen sponge with allogenic skin fibroblasts, the wound process is at the stage of proliferation with the formation of granulation tissue. In the control group, the wound’s surface is covered with a thick crust consisting of fibrin and dead cells (Fig. 1). Beneath it, there is a thin layer of developing epidermis made of 5–6 raw cells. Nominally they can be divided into basal and spinous layers. The average thickness of the epidermis is 39.73±0.12 microns (Table). In the centermost portions of the wound bounded with inserted silicone rings, the epithelium is absent. Under the surface epithelium, granulation tissue is well developed. Collagen fibers are thin and short, lie randomly, and intersect. In between, fibroblast cells are typically active specialized fibroblasts with rounded or oval nuclei. Weak leukocyte infiltration is only present in deep biopsies. CD-3 positive cells, or T-lymphocytes, were not found among them. Granulation tissue is well vascularized. Blood capillaries are numerous, comprehensive, and partly filled with blood.

Experimental group biopsies do not show the presence of collagen sponge, which has wholly resorbed. The thickness of the crust is visibly decreased. In contrast to the control group, the epidermis is thicker, 20.30±0.11 %. The number of cell raws increased up to 5–6, and there appeared signs of granular layer formation (Fig. 2). On the surface of the epidermis, there were traces of cells with basophilic granules. The total surface of the wound is covered with epithelium. Within the granulation tissue, collagen fiber area increases by 36.63±0.12 % in contrast to the control group. Thickenened oxyphilic fibers became more ordered – they lie parallel to each other and to the epidermis, which can be understood as the signs of the initial stage of cicatrix formation. Functional fibroblasts lie between collagen fibers and have an oblong shape with elongated nuclei. Blood vessel area decreased by 54.17±0.2 %. However, single veins in deep layers of the biptic are widened and filled with blood. They are surrounded by leucocytes, among which there are T-lymphocytes (CD-3+ cells) in a negligible quantity. Their index is 4.68±0.01 %.

A three-dimensional hybrid matrix derived from a collagen-resorbable sponge and cultured dermal allogenic fibroblasts can be considered an effective skin substitute for ischemic skin wounds. It is known that the breakdown products of collagen due to the specific interaction of collagen with fibroblasts by feedback
mechanism stimulate the proliferation of fibroblasts and
the biosynthesis of the native collagen, i.e., regeneration
of the connective tissue [11]. By the 10th day of
regeneration, the wound process in an experimental
group had made significant progress compared to the
control one. It is probably caused by more fibroblasts and
extracellular matrix in the form of collagen, which doesn’t
result in potent T-lymphotye input.

Conclusion. By the 10th day, since the simulation of
a persistent ischemic cutaneous wound and its closure
by collagen sponge with allogenic dermal fibroblasts,
it was shown a vast improvement to morphological
characteristics of the wound process second stage.
Bioplate granulation tissue is 36.63±0.12 % high in
collagen fibers and 54.17±0.2 % low in blood vessels in
contrast to the control group, with the signs of fibrotic
scarring being absent in the control group, indicating the
cicatrization onset. Epidermis is 20.3±0.11 % thicker in
contrast to the control group. By then, the collagen sponge
has been wholly resorbed without potent T-Lymphocyte
input with a CD3+ cell index of 4.68±0.01 %.

Informed consent. The study was approved by the
Local Ethics Committee of V. I. Vernadsky Crimean
Federal University. The basic rules for the maintenance
and care of experimental animals corresponded to the
standards given in the Order of the Ministry of Health
of the Russian Federation № 708n of 23.08.2010
«On Approval of the Rules of Laboratory Practice in the
Russian Federation», the ethical principles established by
the European Convention for the Protection of Vertebrates
Used for Experimental and Other Scientific Purposes
(adopted in Strasbourg on 18.03.1986 and confirmed
in Strasbourg on 15.06.2006). Also, the experiment was
carried out in compliance with all principles of humanity
contained in the directive of the European Community
(86/609/EC).

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Fig. 2. Bioplate of mouse skin in experimental group:
1 – crust; 2 – epidermis; 3 – collagen fibers; 4 – blood capillary;
5 – leucocytes infiltration. Stained by hematoxylin and eosin.
Magnification: 400
The study determined the effect of silver nanoparticles (AgNPs) in the treatment of purulent wounds with negative pressure instillations (NPWTi) on the parameters of the antioxidant system of blood and exudate. The study was conducted on 72 laboratory animals, which were divided into four equal groups. In contrast, in 3 groups, it was performed by modeling a purulent wound with its further treatment in various ways. In group 1, the traditional treatment method was used under a bandage with a levomekol; in group 2, wound treatment was carried out using a preparation with AgNPs; in group 3, the NPWTi method was used. In group 4 (control), modeling of a purulent wound was not performed. During the experiment, a study was made of the parameters of the enzyme link of antioxidant protection (catalase and superoxide dismutase), as well as the total antioxidant activity due to the content of low molecular weight substrates. It should be noted that only in animals of group 3, the indicator of antioxidant activity of the blood reached the values of the control group on the 12th day. In contrast, in group 2 it remained significantly reduced by 13.2 %. The data obtained indicate the ability of AgNPs to increase the prooxidant load in the wound area mainly in the first six days, with the development of an imbalance in the work of enzymes of the 1st and 2nd lines of antioxidant protection. This may indicate the advisability of the combined use of AgNPs and NPWTi in treating the first phase of the wound process.

Keywords: wound process, silver nanoparticles, negative pressure instillations, wound treatment, oxidative stress, catalase, superoxide dismutase