UDC 611-018.2+611-013:616-003.93
DOI – https://doi.org/10.14300/mnnc.2023.18037
ISSN – 2073-8137

T-LYMPHOCYTES AND MORPHOLOGY OF ISCHEMIC CUTANEOUS WOUND OF MICE WITHIN COLLAGEN SPONGE WITH ALLOGENIC DERMAL FIBROBLASTS TRANSPLANTATION

Baranovskiy Yu. G., Shapovalova E. Yu., Boyko T. A., Lugin I. A., Harchenko S. V., Baranovskiy A. G., Kubyshkin A. V.

Medical Academy named after S. I. Georgievsky, Simferopol, Russian Federation

Т-ЛИМФОЦИТЫ И МОРФОЛОГИЯ РЕГЕНЕРАТА ИШЕМИЗИРОВАННОЙ РАНЫ КОЖИ МЫШЕЙ В УСЛОВИЯХ ТРАНСПЛАНТАЦИИ В РАНУ КОЛЛАГЕНОВОЙ ГУБКИ С АЛЛОГЕННЫМИ ДЕРМАЛЬНЫМИ ФИБРОБЛАСТАМИ

Ю. Г. Барановский, Е. Ю. Шаповалова, Т. А. Бойко, И. А. Лугин, С. В. Харченко, А. Г. Барановский, А. В. Кубышкин

Медицинская академия им. С. И. Георгиевского, Симферополь, Российская Федерация

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 allofibroblasts, 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 коллагеновой губки с дермальными аллогенными фибробластам для стимуляции ранозаживления. Шестнадцать половозрелых мышей линии С57/В1 были разделены на две равнозначные группы: контроля и экспериментальную. В динамике к 10-м суткам выявлена высокая эффективность метода стимуляции ассоциацией коллагеновой губки с аллофибробластами, позволяющего существенно улучшить морфологические характеристики биоптатов и не вызывающего аутоиммунной реакции на трансплантат.

Ключевые слова: ишемизированная рана кожи, коллагеновая губка, фибробласты, Т-лимфоциты

For citation: Baranovskiy Yu. G., Shapovalova E. Yu., Boyko T. A., Lugin I. A., Harchenko S. V., Baranovskiy A. G., Kubyshkin A. V. T-LYMPHOCYTES AND MORPHOLOGY OF ISCHEMIC CUTANEOUS WOUND OF MICE WITHIN COLLAGEN SPONGE WITH ALLOGENIC DERMAL FIBROBLASTS TRANSPLANTATION. *Medical News of North Caucasus*. 2023;18(2):169-172. DOI – https://doi.org/10.14300/mnnc.2023.18037

Для цитирования: Барановский Ю. Г., Шаповалова Е. Ю., Бойко Т. А., Лугин И. А., Харченко С. В., Барановский А. Г., Кубышкин А. В. Т-ЛИМФОЦИТЫ И МОРФОЛОГИЯ РЕГЕНЕРАТА ИШЕМИЗИРОВАННОЙ РАНЫ КОЖИ МЫШЕЙ В УСЛОВИЯХ ТРАНСПЛАНТАЦИИ В РАНУ КОЛЛАГЕНОВОЙ ГУБКИ С АЛЛОГЕННЫМИ ДЕРМАЛЬНЫМИ ФИБРОБЛАСТАМИ. *Медицинский вестник Северного Кавказа*. 2023;18(2):169-172. DOI – https://doi.org/10.14300/mnnc.2023.18037

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

cattle) [5], birds (duck legs) [6], and fish [7]. 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 allogenous dermal fibroblasts.

Material and Methods. The researchers used 4–6 month laboratory white mice of line C57/B1 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 (Lonza) 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 bioptic 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 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 3–4 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.

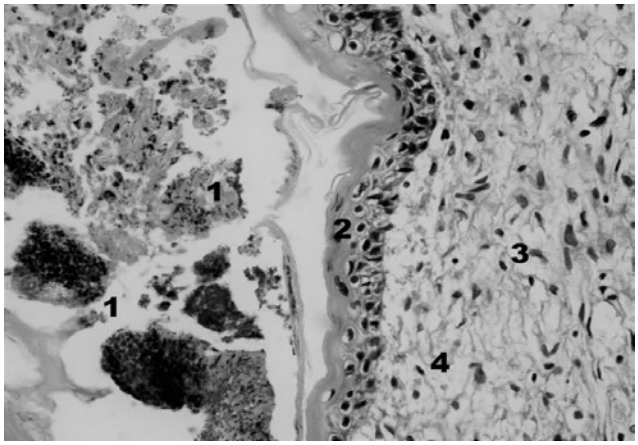

Fig. 1. Bioptate of mouse skin in control group: 1 – crust; 2 – epidermis; 3 – blood capillary; 4 – collagen fibers. Stained by hematoxylin and eosin. Magnification: 400

Table

Quantitive caracteristics of bioptate components of control and experimental groups

Bioptates	Thickness of epidermis (microns)	Dermis area on slices (microns)	Dermal vessels area (%)	Collagen fiber area in dermis (%)
Control group	39,73±0,12	43355,47±1,20	0,52±0,01	21,68±0,12
Experimental group	49,85±0,15	56054,14±1,05	0,96±0,02	34,21±0,12

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. Thickened 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 bioptic 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-lymphocyte input.

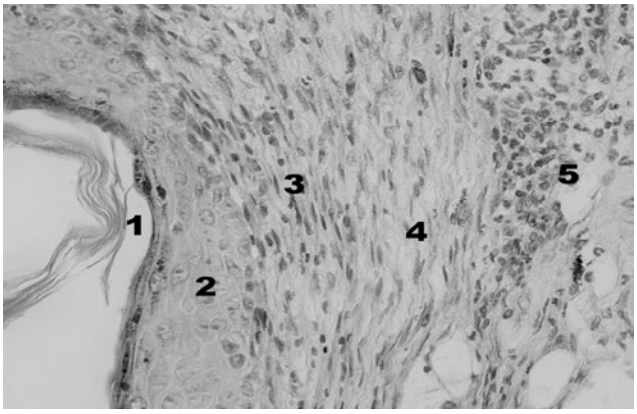

Fig. 2. Bioptate of mouse skin in experimental group:
1 – crust; 2 – epidermis; 3 – collagen fibers; 4 – blood capillary; 5 – leucocytes infiltration. Stained by hematoxylin and eosin. Magnification: 400

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. Bioptate 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).

Disclosures: The authors declare no conflict of interest.

References

1. Guest J. F., Fuller G. W., Vowden P. Venous leg ulcer management in clinical practice in the UK: costs and outcomes. *Int. Wound J.* 2018;15(1):29-37. https://doi.org/10.1111/iwj.12814
2. Nair R. P. Contribution of Fibroblasts to the Mechanical Stability of In Vitro Engineered Dermal-Like Tissue Through Extracellular Matrix Deposition. *Biores. Open Access.* 2014;(5):217-225.
3. Volk S. W., Iqbal S. A., Bayat A. Interactions of the Extracellular Matrix and Progenitor Cells in Cutaneous Wound Healing. *Adv. Wound Care (New Rochelle)*. 2013;(6):261-272.
4. Ramanathan G., Singaravelu S., Muthukumar T., Thyagarajan S., Perumal P. T., Sivagnanam U. T. Design and characterization of 3D hybrid collagen matrixes as a dermal substitute in skin tissue engineering. *Mater. Sci. Eng. C. Mater. Biol. Appl.* 2017;72:359-370. https://doi.org/10.1016/j.msec.2016.11.095
5. Parenteau-Bareil R., Gauvin R., Berthod F. Collagen-based biomaterials for tissue engineering applications. *Materials.* 2010;3(3):1863-1887. https://doi.org/10.3390/ma3031863
6. Sultan M. T., Jeong J. Y., Seo Y. B., Lee O. J., Ju H. W. [et al.] Fabrication and characterization of the porous duck's feet collagen sponge for wound healingapplications. *J. Biomater. Sci. Polym. Ed.* 2018;29(7-9):960-971. https://doi.org/10.1080/09205063.2017.1367636
7. Chen J., Gao K., Liu S., Wang S., Elango J. [et al.] Fish Collagen Surgical Compress Repairing Characteristics on Wound Healing Process In Vivo. *Mar. Drugs.* 2019;17(1):E33. https://doi.org/10.3390/md17010033
8. Shekhter A. B., Guller A. E., Istranov L. P., Istranova E. V., Butnaru D. V. [et al.] Morphology of collagen matrices for tissue engineering (biocompatibility, biodegradation, tissue reaction). *Arkhiv patologii*. 2015;77(6):29-38. https://doi.org/10.17116/patol201577629-38
9. Jinno C., Morimoto N., Ito R., Sakamoto M., Ogino S. [et al.] A Comparison of Conventional Collagen Sponge and Collagen-Gelatin Sponge in Wound Healing. *Biomed. Res. Int.* 2016:4567146. https://doi.org/10.1155/2016/4567146
10. Baranovskiy Yu. G., Il'chenko F. N., Shapovalova E. Yu. Method for modeling trophic ulcers in laboratory mice in the experimental model. *Vestnik neotlozhnoy i vosstanovitel'noy khirurgii.* 2016;1(2):259-261.
11. Shapovalova Ye. Yu., Boyko T. A., Baranovskiy Yu. G., Morozova M. N., Barsukov N. P. [et al.] Effects of fibroblast transplantation on the content of macrophages and the morphology of regenerating ischemic cutaneous wounds. *Int. J. Biomed. Res*. 2017;4:302-306. https://doi.org/10.21103/Article7(4)_OA6
12. Kova L. V. P., Viktorov A. P. Compendium 2006 – Medicinal Products. K.: Morion. 2006:1126.
13. Shapovalova E. Yu., Boyko T. A., Baranovskiy Yu. G., Harchenko S. V., Yunsi G. A. Morphological characteristics of the healing of an ischemic experimental wound on day 12 after the application of auto- and heterofibroblasts and dermal equivalent. *Mezhdunarodnyy nauchno-issledovatel'skiy zhurnal.* 2017;8(3):51-55. https://doi.org/10.23670/IRJ.2017.62.051

About authors:

Baranovskiy Yuriy Gennadievich, PhD, MD, Associate professor, Associate Professor of the Department of Surgery № 2;
tel.: +79787196605; e-mail: baranovskiy_yura@mail.ru; ORCID: 0000-0002-7044-1122

Shapovalova Elena Yur'evna, MD, PhD, Professor, Head of the Department of Histology and Embryology;
tel.: +79787657196; e-mail: Shapovalova_L@mail.ru; ORCID: 0000-0003-2544-7696

Boyko Tatyana Anatolievna, MD, PhD, Associate professor, Associate professor of Department of Histology and Embryology;
tel.: +79787198488; e-mail: tatyana_boyko_5@mail.ru; ORCID: 0000-0002-9627-4051

Lugin Igor Anatolievich, MD, PhD, Associate professor, Associate professor of Department of Histology and Embryology;
tel.: +79788102168; e-mail: iglug@list.ru; ORCID: 0000-0002-9297-97038

Harchenko Svetlana Vladimirovna, MD, PhD, Associate professor, Associate professor of Department of Histology and Embryology;
tel.: +79788649204; e-mail: kcharchenkos@mail.ru; ORCID: 0000-0003-2602-0504

Baranovskiy Aleksey Gennadievich, Department of Histology and Embryology;
tel.: +79787454483; e-mail: baranovskiy_alexey@mail.ru; ORCID: 0000-0001-6995-3975

Kubyshkin Anatoliy Vladimirovich, MD, PhD, Professor, Head of the Department of General and Clinical Pathology Physiology;
tel.: +79780280111; e-mail: kubyshkin_av@mail.ru; ORCID: 0000-0002-1309-4005

UDC 616-001.4:546.57-08
DOI – https://doi.org/10.14300/mnnc.2023.18038
ISSN – 2073-8137

DYNAMICS OF INDICATORS OF THE PROOXIDANT-ANTIOXIDANT SYSTEM IN THE BLOOD AND EXUDATE UNDER THE TREATMENT OF PURULENT WOUNDS WITH NEGATIVE PRESSURE AND SILVER NANOPARTICLES

Malyshko V. V. [1], Basov A. A. [1], Dorohova A. A. [2], Moiseev A. V. [3], Dyakov O. V. [1], Pavlyuchenko I. I. [1], Storozhuk A. P. [1]

[1] Kuban State Medical University, Krasnodar, Russian Federation
[2] Kuban State University, Krasnodar, Russian Federation
[3] Kuban State Agrarian University, Krasnodar, Russian Federation

ДИНАМИКА ПОКАЗАТЕЛЕЙ ПРООКСИДАНТНО-АНТИОКСИДАНТНОЙ СИСТЕМЫ В КРОВИ И ЭКССУДАТЕ ПРИ ЛЕЧЕНИИ ГНОЙНЫХ РАН ОТРИЦАТЕЛЬНЫМ ДАВЛЕНИЕМ И НАНОЧАСТИЦАМИ СЕРЕБРА

В. В. Малышко [1], А. А. Басов [1], А. А. Дорохова [2], А. В. Моисеев [3], О. В. Дьяков [1], И. И. Павлюченко [1], А. П. Сторожук [1]

[1] Кубанский государственный медицинский университет, Краснодар, Российская Федерация
[2] Кубанский государственный университет, Краснодар, Российская Федерация
[3] Кубанский государственный аграрный университет, Краснодар, Российская Федерация

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

В исследовании определялось влияние наночастиц серебра (AgNPs) в лечении гнойных ран отрицательным давлением с инстилляциями (NPWTi) на показатели антиоксидантной системы крови и экссудата. Исследование проведено на 72 лабораторных животных, которые были разделены на 4 равные группы, при этом в 3 группах выполнялось моделирование гнойной раны с дальнейшим ее лечением различными способами. В группе 1 использовали традиционный метод лечения под повязкой с мазью, содержащей левомеколь; в группе 2 лечение ран осуществляли с помощью препарата с наночастицами серебра; в группе 3 применяли метод NPWTi. В группе 4 (контрольной) моделирование гнойной раны не выполнялось. В ходе эксперимента было проведено изучение показателей ферментного звена антиоксидантной защиты (каталазы и супероксиддисмутазы), а также общей антиоксидантной активности, обусловленной содержанием низкомолекулярных субстратов. Следует отметить, что только у животных группы 3 показатель антиоксидантной активности крови достигал значений контрольной группы на 12 сутки, тогда как в группе 2 он оставался достоверно сниженным на 13,2 %. Полученные данные свидетельствуют о способности AgNPs увели-