Investigation of the dynamics of the healing of skin wounds in rats under the influence of low-intensity electromagnetic fields of endogenous origin in experiment

N.T. Salia
(NTSSSH named after A.N.Bakulev, Moscow)

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NT Salia
AN Bakulev SCCVS (Moscow, Russia)

SUMMARY

The work is devoted to the study of the effectiveness of exposure to low-intensity electromagnetic fields of endogenous origin (endogenous bioresonance therapy) in the healing of postoperative wounds in the experiment. Planimetric, cytological and immunohistochemical studies were carried out. It has been established that bioresonance therapy accelerates and stimulates the wound healing process, both the epithelialization process and the process of cell proliferation in the wound area.

Key words: low-intensity electromagnetic fields, bioresonance therapy,postoperative wounds, mitotic activity, proliferative activity.

RESUME

The efficiency of use of low-intensity electromagnetic fields of endogenous origin (endogenous bioresonance therapy) was studied in experiment on the model of postoperative wound healing. Planimetric, cytological and immunohistochemical studies were conducted. We have demonstrated that bioresonance therapy accelerates and stimulates the healing process, both the process of epithelialization, and the process of cell proliferation in the wound area.

Keywords: low-intensity electromagnetic fields, endogenous bioresonance therapy, postoperative wounds, mitotic activity, proliferative activity.

Treatment of wounds of various origins remains one of the important problems of modern medicine. Even after operations classified as "clean", the postoperative period is sometimes accompanied by various complications. Therefore, the search for effective methods to accelerate the healing process is relevant at the present time. There are many treatments for postoperative wounds [1-3]. However, from the point of view of modern requirements for the regulation of inflammation and regeneration, neither antibacterial, nor enzymatic, nor vacuum aspiration, nor ultrasonic cavitation, nor treatment of wounds with a pulsating jet, etc. do not have a direct impact on these processes.

There are data in the literature indicating the possibility of the influence of low-intensity electromagnetic radiation on the processes of tissue regeneration [4–5]. Thus, according to the literature [7–9], studies are carried out, possible mechanisms of the effect of low-intensity electromagnetic radiation on biological objects are discussed. Disclosure of the mechanisms of regulation of bioenergetic processes occurring in the cellular structures of the body under the influence of low-intensity electromagnetic radiation will undoubtedly contribute to the development of clear indications for appropriate therapy in various pathological conditions.

There are reports in the literature on the effect of low-frequency electromagnetic fields on biological objects [7–11], but there are no publications reporting on the use of low-intensity electromagnetic fields of endogenous origin in the treatment of postoperative wounds. In foreign literature, there are isolated works devoted to the study in the experiment of low-intensity electromagnetic fields of endogenous origin.

[12-14]. Meanwhile, electromagnetic fields of both endogenous and exogenous origin play an important role in biological processes in the human body [15, 16].

The method of endogenous bioresonance therapy (BRT) [18–20] is one of the options for therapy with low-intensity electromagnetic fields of endogenous origin.

Experimental substantiation of the use of BRT in the treatment of wounds and the prevention of disorders of their healing opens up new perspectives in the fight against disorders of the wound process in the early postoperative period, provides prerequisites for improving the quality of postoperative management of patients, including cardiac surgery, and reducing the likelihood of postoperative complications.

Purpose of the study

Estimate the change healing rate wounds, conduct cytological and immunohistochemical studies of the effect of endogenous bioresonance therapy (BRT) on the healing of wounds in white rats.

Material and research methods

Experimental studies were carried out on 260 sexually mature rats weighing 130–140 g. All animals were divided into control and experimental groups. All animals, both the control and the experimental group, were kept under the same conditions. A 2 x 2 cm flap of skin was cut out from the back of the animals of both groups. The experimental group of animals underwent daily sessions of bioresonance therapy according to strategy 4 with the implementation of exposure to the animals using a device for magnetic therapy "loop" for 7 days for 1 hour ... Within one month, every second day after the operation, the area of wounds was measured in both groups. The results obtained were processed using computer programs (CorelDraw 11 and AutoCAD-2002). The reliability of the results was assessed by the Student criterion ($p < 0.05$).

To assess the mitotic (MI) index of skin cells, 10 animals from the control and experimental groups were selected, three hours before sacrifice, a colchicine solution was injected intraperitoneally (1 μg / g weight). The material (pieces of skin tissue from the wound area) was fixed in a 4% formalin solution prepared in Na, K-phosphate buffer (pH 7.2–7.4). Fixed pieces of tissue were embedded in paraffin, sections of 5–7 μm thick were prepared, which were then stained with hematoxylin-eosin. The preparations were examined under a LOMO light microscope (magnification 90 × 10). The mitotic index in ppm (‰) was determined by counting at least 5000 cells for each case. Individual sections were processed by the immunohistochemical method using monoclonal antibodies to the Ki67 protein. To assess the epithelialization process, we used antibodies to a specific marker protein of keratinocytes (pan Cytokeratin antibody, PCK-26, ab6401). The more euthanized animals were carried out under ether anesthesia. The reliability of the data obtained was assessed using the Student's test.

Results and discussion

Measurement of the surface of skin wounds in dynamics in animals that underwent bioresonance exposure showed that wound healing in this group begins earlier than in animals of the control group.

The dynamics of the wound healing process in animals of the experimental and control groups is shown in Fig. 1. As can be seen from the data presented in the figure, the dynamics of the healing process of skin wounds in the control and experimental groups until the 13th day after the application of skin wounds is not much different. But, starting from the 13th day, the difference in the size of skin wounds in the experimental group significantly differs from the control ($p < 0.05$). The healing process in the control group begins on day 18 and ends on day 29. As you know, 15–20 days correspond to the third phase of wound healing - the phase of scar formation and its epithelialization [22, 23]. In the experimental group, the healing process of skin wounds is completely completed by 23 days after the application of the skin wound.

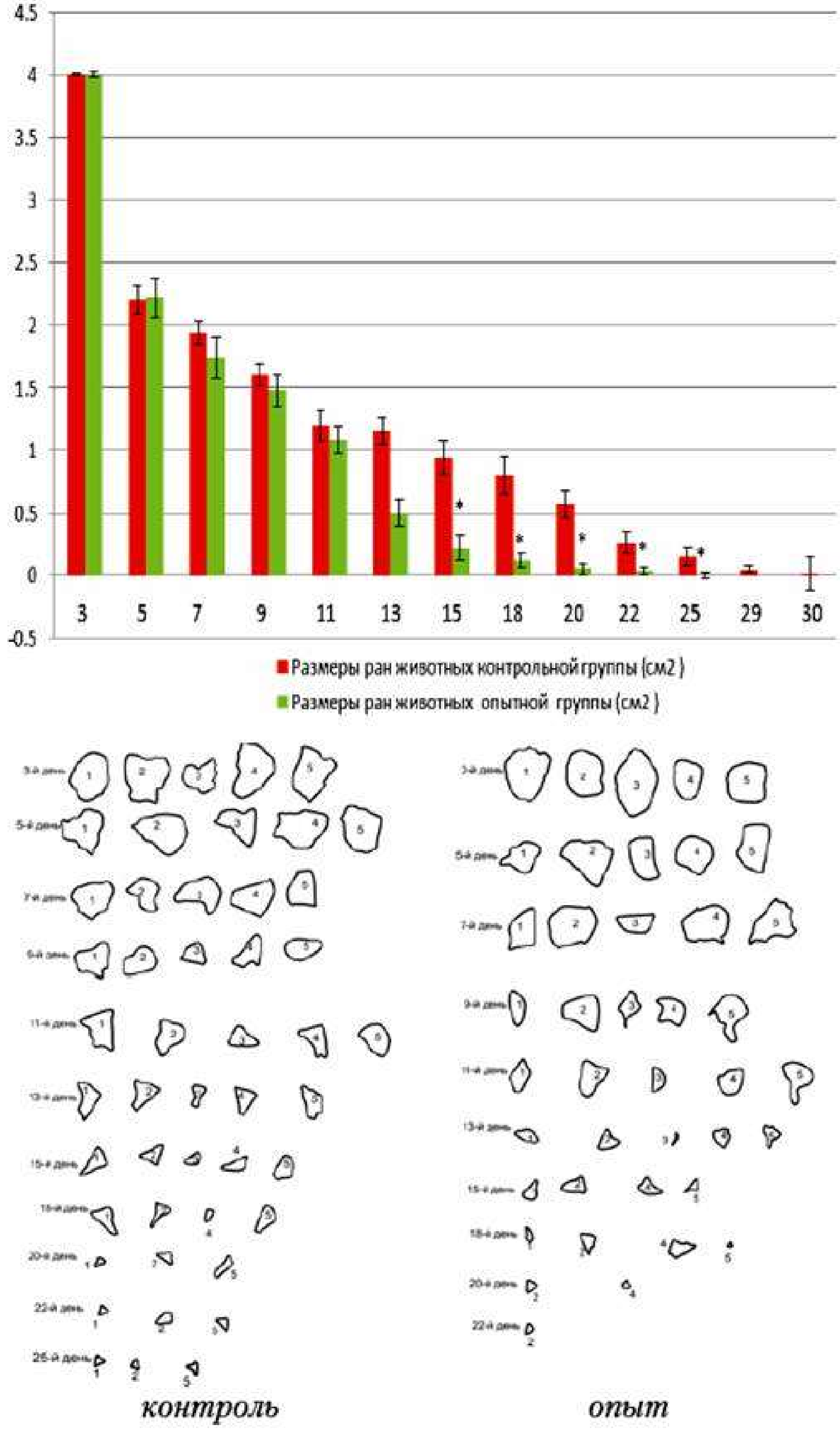
The results of the study indicate that the wound healing process in the experimental group ends in an average of 20 ± 2.5 days, while in the control group - in 26 ± 3 days.

The results of histological studies obtained by us are consistent with the results authors [24] on the acceleration of wound healing processes under the influence of electromagnetic waves of the millimeter range.

In fig. Figures 2a, 2b show the results of the effect of BRT on changes in the MI of skin cells of white sexually mature rats in dynamics. As can be seen from the results of the study presented in the figure, a surge in mitosis in animals of the experimental group is observed already 24 hours after the operation. In addition, by the 28th hour, mitotic activity reached its peak (the first peak of mitotic activity) and amounted to almost 45 ppm ($p < 0.001$). After the first burst, a decline in mitotic activity was observed. The second, however, a relatively small burst of mitosis was again detected three days after resection of the skin area. It should be noted that the mitotic activity of cells in the area of wounds of animals of the control group did not change for 4 days and averaged 10 ppm ($p < 0.001$).

Conducted in parallel with this, the assessment of the proliferative activity of skin cells by determining the activity of the marker protein in the wound area of adult white rats showed that the marker protein of proliferation Ki67 is manifested in the interphase cell only in the nucleus, while during mitosis it is found on the chromosomes. In a number of works [25], based on the study of the localization of cytokeratins 5 and 13, the dynamics of re-epithelialization of skin wounds and the distribution of the Ki 67 antigen proliferation marker in them were traced, which made it possible to detect proliferating fibroblasts starting from 1.5 days after injury.

In fig. 2d shows the curves reflecting the number of cells stained for the marker protein of proliferation in dynamics. As can be seen from the data presented in the figure, the number of skin cells stained for the marker protein of proliferation Ki67 from the area of wounds of white mature rats of the experimental group during the first day significantly exceeds the corresponding indicator of animals in the control group ($p < 0.05$).



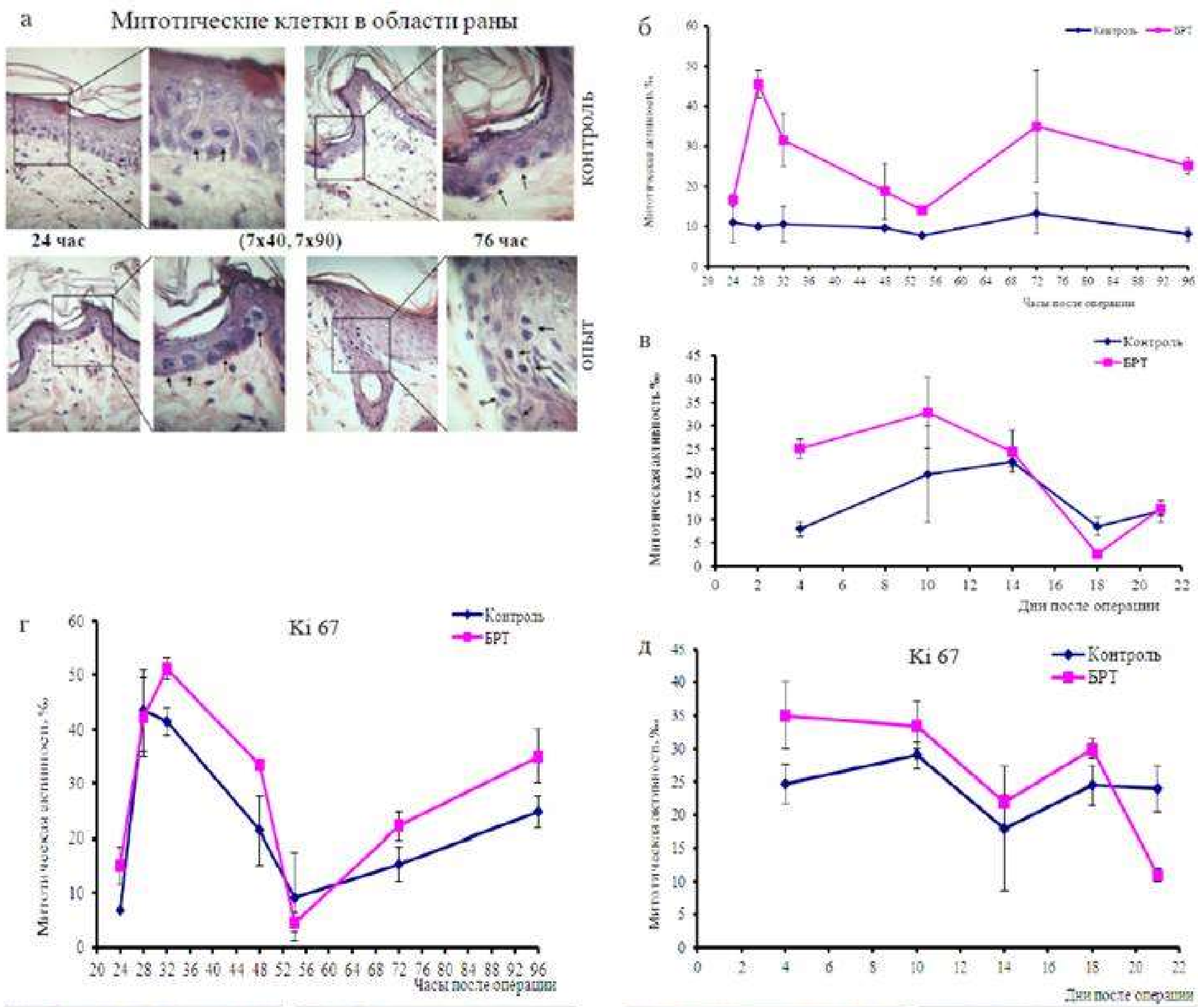
Rice. 1. Dynamics of changes in the size of skin wounds as a result of bioresonance effects. *$p < 0.05$.

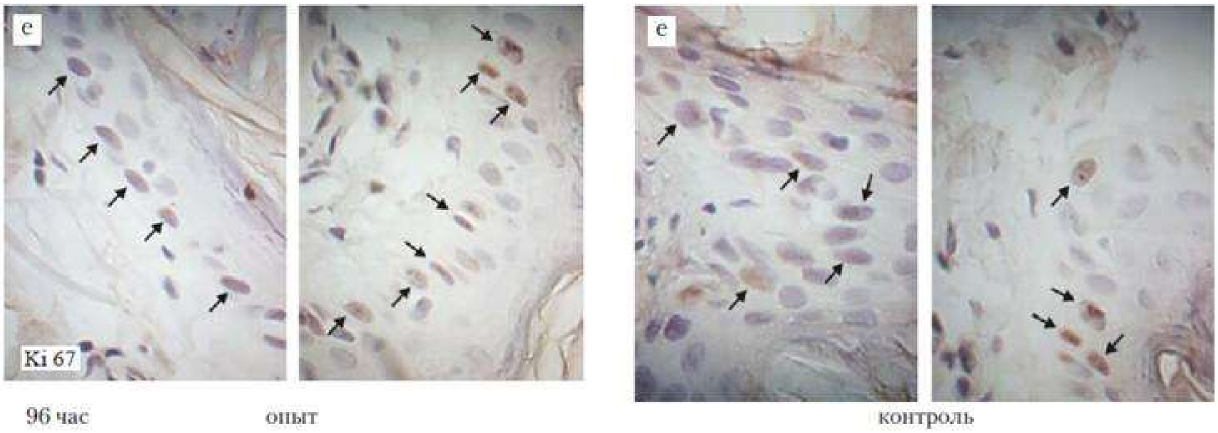
If we compare the curves shown in Fig. 2b and 2d, which reflect the proliferative activity of cells in the wound area, it can be seen that in both cases two peaks appear corresponding to the number of stained cells by the 32nd ($p < 0.001$) and 96th hour ($p < 0.05$) and mitotic cells by the 28th and 72nd hours.

In the dynamics of observation of the change in the mitotic index of skin cells from the 4th to the 21st day after surgery (Fig. 2c, 2e), it was shown that the difference between the MI indices of animals the control and experimental groups is not reliable, from which it follows that ten days after the operation, the effect of BRT sessions on the proliferative activity of cells is not observed ($p > 0.3$).

Similarly to mitotic activity, ten days after surgery, the effect of BRT sessions on the number of Ki 67 positive cells was also not observed (Figs. 2e, 2f). We also evaluated the process of wound healing in animals on tissue sections obtained from the wound area (Fig. 3). In fig. 3 shows micrographs of paraffin sections of animals of the control and experimental groups, respectively. According to the data obtained, skin regeneration in the control group on the 21st day is still ongoing. As for the animals of the experimental group, the process of skin regeneration, as shown in the micrographs, after the BRT sessions proceeds faster. As seen from Fig. 3a, the epithelialization process in the experimental group was completely completed on day 21, which once again confirms the stimulating effect of BRT on the wound healing process.

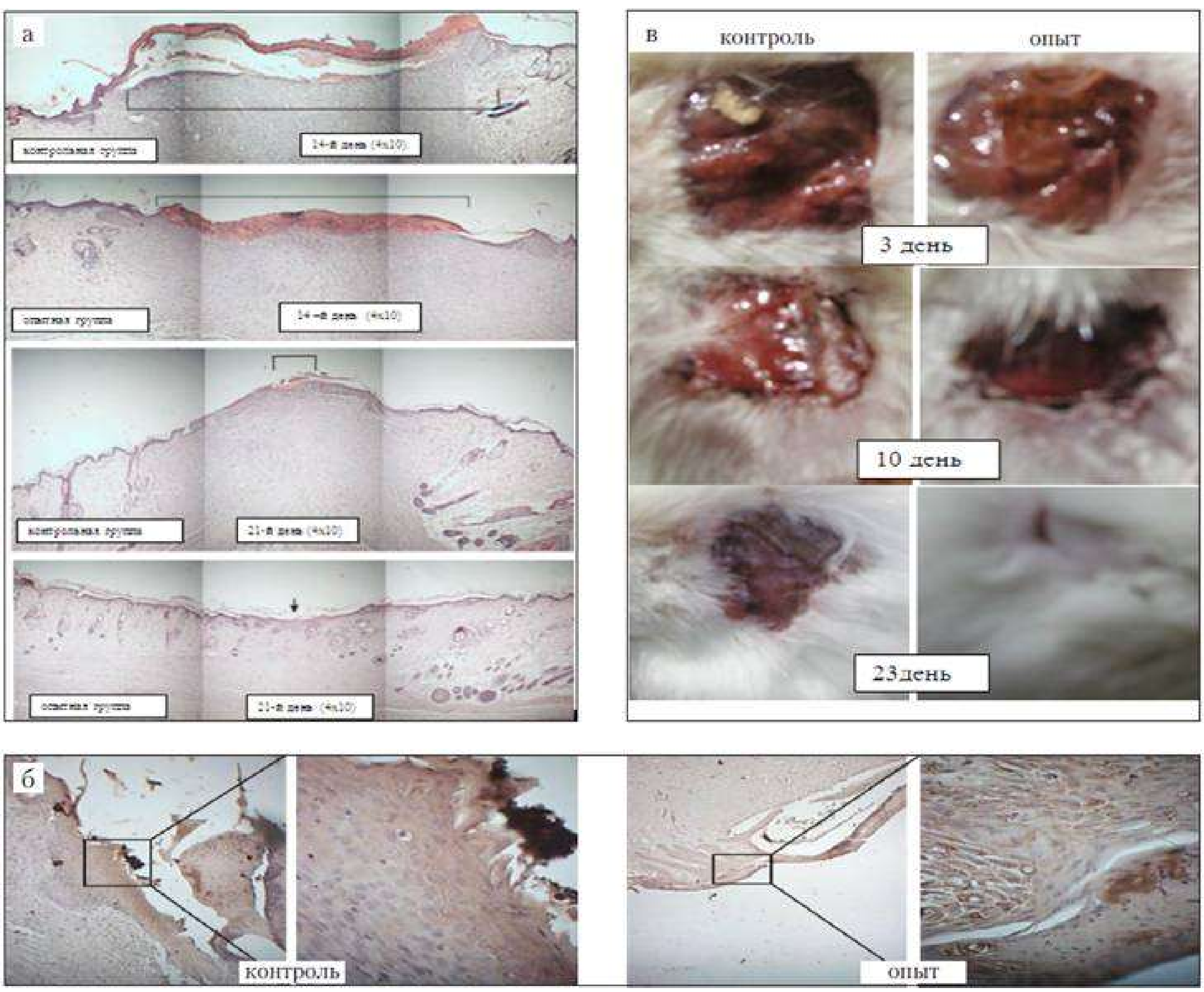
An indicator of success in wound treatment is the epithelialization of the wound surface. The high rate of epithelialization of wounds is provided by three processes: migration, division and differentiation of cells [26]. This process is a series of sequential events including mobilization, migration, mitosis and cellular differentiation of epithelial cells. Regeneration of the epithelium is an essential point in tissue restoration, provides an effective barrier against bacterial invasion, the necessary function and appearance. This process includes the mobilization of basal cells from their attachment to the dermis, migration to the site of injury, mitotic proliferation, and replacement of antecedent cells. To assess the epithelialization process, antibodies to a specific marker protein of keratinocytes were used.





Rice. 2. Dynamics of the wound healing process as a result of exposure to low-intensity electromagnetic fields of endogenous origin (a, b, c - dynamics of changes in the mitotic index (MI) of skin cells of white sexually mature rats; d, e, f - dynamics of changes the number of skin cells stained for the marker protein of proliferation (Ki 67)).

Studies have shown that the cells of stratified squamous epithelium move from the edges of the wound, i.e. there is a normal epithelialization process. The positive effect of BRT on the wound healing process manifests itself 96 hours after surgery (Fig. 3b). In fig. 3b clearly shows the newly formed stratified squamous epithelium in the wound area of the animals of the experimental group.



Rice. 3. Dynamics of wound healing as a result of bioresonance effects in sexually mature whites

rats. a - microphoto-paraffin sections of the wound surface of the skin in dynamics; b - migration epithelial cells in the wound area; c - photographs of the wound surface of the skin in dynamics.

conclusions

Based on the experimental studies carried out to study the effect of BRT on the wound healing process, a significant acceleration of the healing process of skin wounds was shown by an average of 6 days. The stimulating effect of BRT on both the epithelialization process and the process of cell proliferation in the wound area was revealed. It was found that a burst of mitosis in animals of the experimental group is observed already 24 hours after the operation, reaching its peak by 28 hours, and is almost 45 ppm.

The results of the studies carried out indicate that bioresonance therapy with low-intensity electromagnetic fields of endogenous origin accelerates the processes of regeneration and reparation in the body.

Literature

1. Konstantine C. Balakatounis and Antonios G. Angoulesc. Low-intensity Electrical Stimulation in Wound Healing: Review of the Efficacy of Externally Applied Currents Resembling the Current of Injury // Open Access Journal of Plastic Surgery. Committed to the free exchange of knowledge in a global community. - 2008. - V.8. Published May, 16.

2. Luther C. Kloth, PT. Electrical Stimulation for Wound Healing: A Review of Evidence From In Vitro Studies, Animal Experiments, and Clinical Trials // LOWER EXTREMITY WOUNDS-2005. –V.4 (1). - R. 23–44.

3. Naomi M Shupak. Therapeutic Uses of Pulsed Magnetic-Field Exposure // A Review. The radio Science Bulletin. - 2003 .-- No. 307.

4. Gassanov L.G., Zemskov V.S., Kovalchuk A.I. and others. The impact of low-intensity electromagnetic radiation on the course of the wound process // Electronic industry. - 1988. - Issue. 8. - P. 20–22.

5. Polyakov A.I., Petrenko Yu.M., Zubkov B.A., Balakireva L.Z. Stimulating action millimeter radiation of low intensity on the wound process // MIS RT. - 1999. - No. 8. - P. 9.

6. Tribrat N.S., Chuyan E.N., Ravaeva M.Yu. Influence of electromagnetic radiation of various range on the processes of microcirculation. Scientific notes of the Tavrichesky National University named after IN AND. Vernadsky Series "Biology, Chemistry". - Volume 22 (61). - 2009. - No. 4. - P. 182–201.

7. Bingi V.N., Rubin A.B. Fundamental problem of magnetobiology. Biomedical technologies and electronics (2-4): 63-76, 2007.

8. Shahin Ahmadian, Saeed Rezaei Zarchiand Bahram Bolouri. The Effects of Extremely Low Frequency Pulsed Electromagnetic Field on Collagen Synthesis of Rat Skin // Biochemical and Histoligical Approach. Iranian Biomedical Journal. - January, 2006 .-- 10 (1). - P. 33–38.

9. Bassett CAL Low Energy Pulsing Electromagnetic Fields Modify Biomedical Processes // Bio Essays Vol. 6, No. 1 CHALLENGES.

10. Morell F. Die MORA-Therapie - Therapie mit korpereigenen Schwingungen // Friesenheim, Med-Tronic, 1978.

11. Morell F. MORA-Therapie, Patienteneigene und Farblichtschwingungen Konzeptund Praxis. - Heidelberg: KarlF. Haug-Verlag, 1987.

12. Fedorowski A., Steciwko A., Rabczynski J. Influence of low-frequency electromagnetic field, on growth of endogenous Morris hepatoma and its metastatic ability // Med Sci Monit - 1998. - No. 4 (5). - P.765-773.

13. Fedorowski A., Steciwko J. Rabczynski. Serum cathepsin B activity during regression of Morris hepatoma 5123 DA // Med Sci Monit. - 2004. - No. 10 (5). - P. 144–150.

14. Fedorowski A., Steciwko. J. Rabczynski Influence of low-frequency electromagnetic field generated by transformation of endogenous Morris hepatoma field on its growth and metastatic ability (I). // J. Med ScMonit. - 1997. - No. 3 (3). - P. 336-341.

15. Min Zhao Electrical fields in wound healing — An overriding signal that directs cell migration

Seminars in Cell & Developmental Biology 20 (2009) 674–682.

16. Aihua Guo1,2, Bing Song1,3, Brian Reid1,3, Yu Gu1,3, John V. Forrester1, Colin AB Jahoda2 and Min Zhao Effects of Physiological Electric Fields on Migration of Human Dermal Fibroblasts // Journal of Investigative Dermatology (2010) 130, 2320-2327.

17. Funk RHW et al. Electromagnetic effects - From cell biology to medicine // Progress in Histochemistry and Cytochemistry 43 (2009) 177-264.

18. Gotovsky M.Yu., Perov Yu.F., Chernetsova LV. Bioresonance therapy. 2nd ed. Revised idop. - M.: IMEDIS, 2010.

19. Brugemann H. Bioresonanztherapie. Grundlagen und Praxis der weiterentwickelten Therapie mit patien-teneigenen Schwingungen nach Morell // Erfahrun-gsheilkunde. - 1989. - Bd. 38, H.3a. - S.162-167.

20. Brugemann H. Bioresonanztherapie. Grundlagen und Praxis der weiterentwickelten Therapie mit patienteneigenen Schwingungen nach Dr. Morell. Therapieder Zukunft // Ibid. –1991. - Bd. 40, H.10. - S.674–677.

21. Samokhin A.V., Yu.V. Gotovsky. Electropuncture diagnostics and therapy according to the method of R. Voll // 5th stereotyped edition. - M .: IMEDIS, 2007.

22. Serov V.V., Shekhter A.B. Connective tissue. - M .: Medicine, 1981 .-- 312 p.

23. Nikolaev A.B., Shakhtmeister L.Ya., Shakhter A.B. Experimental and clinical aspects reparative processes and methods of their stimulation. - M .: MMI im. Sechenov, 1977 .-- S. 161 - 167.

24. Bessonov A.E., Chemeris N.K., Gapeev A.B., Shibaev N.V., Sadovnikov V.B., Lushnikov K.V., Medvedev N.I., Shumilina Yu.V. Reparative tissue regeneration under the influence of electromagnetic waves of millimeter, infrared and part of the visible range, generated by the "MINITAG" therapeutic device. http://infomedcenter.ru/doc/nauka/1.htm

25. Kharin G.M., Chelyshev Yu.A., Dzhordzhikia T.R., Sitkina K.V., Shakirova A.Z. Immunohistochemical analysis of the dynamics of healing of skin wounds. Almanac of Forensic Medicine 2003 №2. http://www.medline.ru/monograf/sudmed/index2.shtml#contents

26. Lipatov V.A. Pathogenesis of the wound process and approaches to the treatment of purulent wounds. http://www.medicus.ru/surgery/spec/?cont=article&art.

Author's address
Ph.D. Salia N.T.
NTSSSH them. A.N. Bakuleva (Moscow)
natsalia@mail.ru

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