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Preclinical Trials of Distant Non-Invasive Electromagnetic Therapy Accelerating the Recovery of Infected Wounds in Animal Models

Lyubov Germanovna Aistova, Anastasiya Olegovna Fyodorova, Elena Vladimirovna Bondarchuk, Alexey Gennadievich Vaganov*, Ekaterina Anatolyevna Galkina, Gregory Arnoldovich Flaks, Ahmed Dzonovich Aslanov, Rustam Mikhailovich Kalibatov, Murat Arsenovich Gotyzhev

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Abstract

The purpose of this preclinical trial is to investigate the acceleration of infected wound healing processes in laboratory animals using weak, non-ionizing, non-thermal electromagnetic fields applied through a non-invasive electromagnetic therapy. The experiment was conducted at the Far East State Agrarian University between June 2023 and December 2024, involving 14 common rabbits. After inducing infected skin wounds, the rabbits were randomly divided into two equal groups on the 5th day. The control group received local wound treatment with saline solution only, while the experimental group underwent remote, non-invasive electromagnetic therapy. The experimental procedure continued for 17 days, followed by a 30-day observation period. Clinical blood analyses and wound exudate cultures were performed. Wound regeneration was evaluated using planimetric methods and standard statistical analysis. On day 5, leukocytosis exceeded the norm by 3.0% in the control group and by 68% in the experimental group. On day 7, the wound size had reduced by 1.0 cm in three control animals, while five animals in the experimental group showed reductions of between 0.5 and 1.0 cm. By day 9, normothermia was observed in the experimental group, whereas one rabbit in the control group exhibited hyperthermia. By day 14, no wound microflora was detected in the experimental group, unlike in the control group. Thrombocytosis was noted in the experimental group throughout the study, ultimately exceeding baseline levels by 71%. Remote non-invasive electromagnetic therapy can serve as an auxiliary method of wound regeneration, providing both local and systemic immunostimulating effects.

Keywords: Non-invasive electromagnetic therapy, Weak impulse non-ionizing non-thermal electromagnetic fields, EMF, PEMF, Infected wounds, Preclinical trials

Lyubov Germanovna Aistova, Anastasiya Olegovna Fyodorova
Department of Pathology, Morphology and Physiology, Faculty of Veterinary Medicine, Animal Science and Biotechnology, Far Eastern State Agrarian University, Blagoveshchensk-na-Amure, Russian Federation.

Elena Vladimirovna Bondarchuk, Ekaterina Anatolyevna Galkina, Gregory Arnoldovich Flaks
Scientific Center, Joint-stock Company «Concern GRANIT», Moscow, Russia.

Alexey Gennadievich Vaganov*,
Department of Hospital Surgery, Medical Academy, Federal State Budgetary Educational Institution of Higher Education "Kabardino-Balkarian State University named after H.M. Berbekov", Nalchik, Russia.
Department of International Medical Education, Federal State Budgetary Educational Institution of Higher Education "Kadyrov Chechen State University", Grozny, Chechen Republic, Russia.

Ahmed Dzonovich Aslanov, Rustam Mikhailovich Kalibatov, Murat Arsenovich Gotyzhev
Department of Hospital Surgery, Medical Academy, Federal State Budgetary Educational Institution of Higher Education "Kabardino-Balkarian State University named after H.M. Berbekov", Nalchik, Russia.

***E-mail:** aleksejvaganov4@gmail.com

Introduction

The development of new methods for treating infected wounds of various etiologies is a pressing challenge in modern surgical practice. Suppuration accounts for up to 40% of complications in general surgery, and postoperative mortality from purulent infections can reach as high as 42% (Heal *et al*., 2016; Norman *et al*., 2016; Peel *et al*., 2019). The need for effective treatment of purulent trophic wounds is particularly urgent in patients undergoing vascular surgery for obliterating diseases of the lower limbs, especially in the context of diabetes mellitus (Babushkina *et al*., 2017). Currently, incision and drainage is the most widely recognized method for managing infected wounds (Norman *et al*., 2016; Babushkina *et al*., 2017; Peel *et al*., 2019; Li *et al*., 2022; Diao *et al*., 2024; Shen & Bao, 2025).

However, this method has notable drawbacks. The use of tampons and drains—particularly when applied for prolonged periods—can damage delicate granulation tissue and lead to secondary infections [(Kazarian *et al*., 2013; İlhan *et al*., 2022). The degree of wound suppuration depends on both the concentration of the pathogen and its sensitivity to antimicrobial agents, as well as the patient's overall immune reactivity (Kazarian *et al*., 2013; Heal *et al*., 2016;

Li *et al*., 2022). Recently, the influence of low-frequency electromagnetic fields (EMFs) on the treatment of infected wounds has gained interest. Studies by various authors have shown that the effects of EMF exposure vary depending on the frequency and duration of application (Lipatov *et al*., 2001; Arndt & Juffmann, 2009; Tiersch *et al*., 2012; Lambert *et al*., 2013; Ağaçkıran *et al*., 2023; Adams & Petruccione, 2024).

In Russian medical literature, the classification of wound healing phases developed at the Central Military Clinical Hospital named after A.A. Vishnevsky is widely accepted (Andreev *et al*., 2022; Aslan *et al*., 2024). The first stage is the inflammation phase, which involves paretic expansion of microcirculatory vessels, followed by exudation, fibrin loss, and the infiltration of neutrophilic leukocytes into the tissue (Amini *et al*., 2018; Belfiore *et al*., 2024). A key event in this phase is the neutrophilic respiratory burst, marked by the release of reactive oxygen species (ROS), which exhibit high cytotoxic activity toward both damaged host cells and bacterial pathogens (Dröge, 2002; Genestra, 2007; Halliwell & Gutteridge, 2007; Uneno *et al*., 2024). Oxygen free radicals accelerate lipid peroxidation in both host cell membranes and bacterial cells (Usselman *et al*., 2016). Research shows that electromagnetic exposure at specific frequencies during this phase increases oxidative stress and enhances macrophage activity, thereby reducing the duration of the inflammatory phase (Bagheri *et al*., 2018; Gurhan *et al*., 2021; Cirik *et al*., 2023).

The second stage is the regeneration phase, characterized by decreased exudation, initiation of fibrinolysis, wound cleansing, and granulation tissue formation (Andreev *et al*., 2022). This stage often overlaps with the third phase—scar formation and epithelial regeneration. EMF exposure during this stage enhances fibrillogenesis and reduces tissue water content (Pouriran *et al*., 2016; Kouhkheil *et al*., 2018; Dastgheib *et al*., 2020; Graefen *et al*., 2023). It also supports the rhythmic processes of connective tissue maturation and epithelialization, accompanied by the resorption of granulation tissue (Dastgheib *et al*., 2020; Dhanasekar *et al*., 2022). At specific EMF frequencies, a marked reduction in wound size can be observed. The core mechanism underlying these EMF effects is believed to involve the EMF-sensitive clustering of spherical nanobubbles (bubstons) in the intercellular fluid (Bunkin *et al*., 2021a, 2021b, 2022; Skeie & Klock, 2023). These structures may influence ion exchange through membrane channels, contributing to cellular responses. Therefore, studying the application of EMF in the treatment of purulent wounds and tissue regeneration is of significant relevance in modern surgery.

The COVID-19 pandemic has further underscored the importance of EMF technologies, which have already been successfully utilized in global healthcare settings. A comprehensive analysis of past studies led researchers to develop sub-noise level remote EMF technologies aimed at suppressing pathogens. A non-invasive electromagnetic therapy based on this technology was successfully tested in randomized controlled clinical trials involving patients with mild to moderate SARS-CoV-2 infections at the COVID hospital of Samara State Medical University. EMF therapy proved to be both safe and effective in 222 patients, according to these clinical trial results (Fatenkov *et al*., 2024; Son & Lee, 2024).

Preclinical trial purpose: to investigate the treatment of infected wounds in model animals (rabbits) using remote and simultaneous exposure to low-frequency electromagnetic fields (EMF) via non-invasive electromagnetic therapy.

Materials and Methods

These non-randomized preclinical studies were conducted at the Faculty of Veterinary Medicine, Animal Science and Biotechnology, Far Eastern State Agrarian University, Blagoveshchensk-on-Amur, Russian Federation, from June 2023 to December 2024. The experiment involved 14 common rabbits, aged 4–5 months, with an average weight of 3–4 kg. A general clinical examination, including body temperature measurements, was performed prior to wound modeling. All values were within physiological norms. The microbiological profile of the skin was analyzed in 4 rabbits following the induction of purulent wounds, which affected approximately 4–5% of the total body surface (ranging in size from 3.5 to 5.5 cm²). On day 5 post-wounding, the animals were randomly divided into two groups (experimental and control), each containing 7 rabbits.

A background study of rabbit skin microflora revealed the presence of *Staphylococcus epidermidis* (25%), *Staphylococcus saprophyticus* (25%), and *Pseudomonas aeruginosa* (50%). These organisms are known permanent skin commensals in rabbits and do not typically cause inflammation in intact skin.

To model infected wounds, an oval incision (3.5-5.5 cm²) was made in the soft tissue using a stencil. The incision area was marked on the interscapular region, and local infiltrative anesthesia with 0.5% Novocaine was administered. The skin and superficial fascia were dissected, and the skin flap, including fur, was turned inward and fixed with a bandage. A purulent process typically develops within 3–5 days. After wound induction, the control group received only local treatment with 0.9% NaCl solution, without additional interventions. The experimental group underwent the same protocol, supplemented by remote non-invasive electromagnetic therapy. The EMF therapy was administered in four 15-minute sessions, each separated by a 15-minute interval, three times per week with a 48-hour interval between treatments, over 14 days. The total experiment duration was 17 days, with an additional 30-day follow-up period. The study protocol was approved by the Bioethics Committee of the Far Eastern State Agrarian University, Russian Federation (Protocol No. 7, dated 2024/04/14).

Throughout the study, wound healing intensity and quality were assessed through clinical blood tests (including leukocytes, neutrophils, lymphocytes, and platelets) and bacteriological analyses of wound cultures on days 5, 14, and 30 following infection. All procedures adhered to the"National General Ethical Principles of Animal Experimentation" and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (March 18, 1986).

Clinical monitoring included the measurement of body temperature and evaluation of local inflammation signs such as edema, purulent discharge, and granulation tissue formation. Wound repair was assessed using planimetric methods on days 1, 4, 7, 10, and 14. The daily wound area reduction percentage was calculated according to the formula:

$$A = ((S - Sn) \times 100) / (S \times t) \quad (1)$$

where:

- *S* is the wound area (mm²) at the previous measurement,
- *Sn* is the wound area (mm²) at the current measurement,
- *t* is the number of days between measurements.

To record the wound reduction rate, the L. N. Popova test (Okhunov *et al*., 2018) was applied. This method calculates the percentage of wound area reduction across the 1st–5th, 5th–10th, and 10th–15th days using the same formula:

$$A_5d = ((S - Sn) \times 100) / (S \times t) \quad (2)$$

For measurement, a sterile polyethylene film was placed over the wound surface to trace the wound contours. These outlines were then transferred to millimeter graph paper, and the enclosed area was calculated. Statistical analysis was performed using the standard tools in Microsoft Excel 2010.

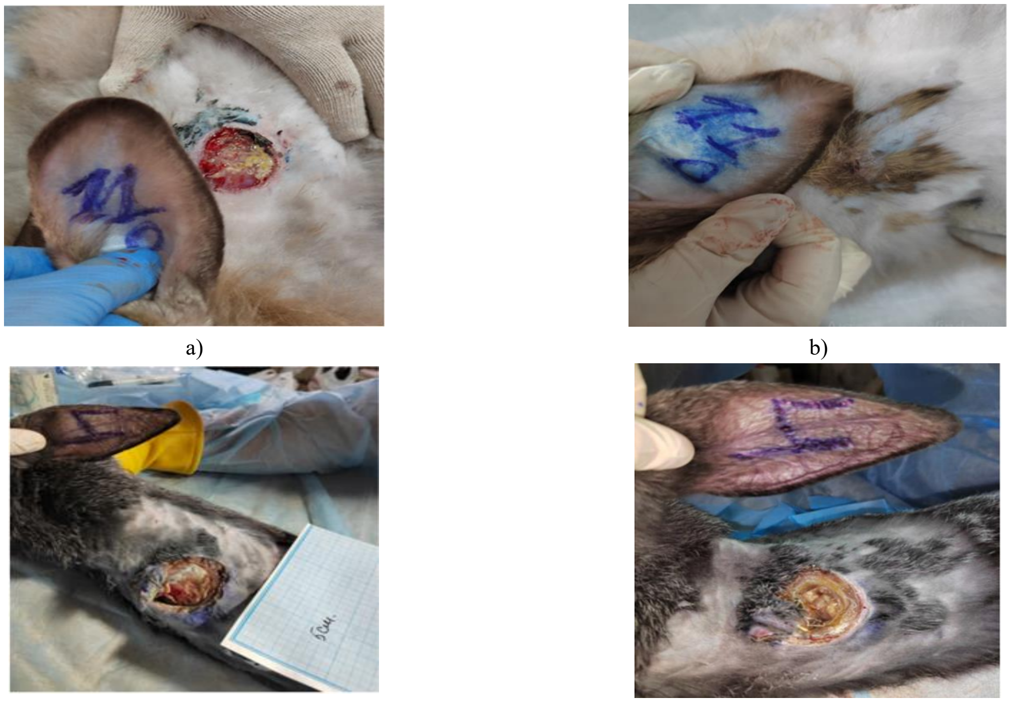
Results and Discussion

Five days after the induction of infected wounds, hyperthermia was observed in one animal from the control group and three animals from the experimental group. On day seven, hyperthermia was present in four rabbits in each group. By day nine, no animals in the experimental group exhibited fever, although one rabbit in the experimental group still had mild hyperthermia. No further increases in body temperature were detected in either group for the remainder of the study **(Table 1)**.

Table 1. Rabbit body temperature during the experiment

##	Animal number	Temperature norm 38.5-39.5 ºC					
		Day 1	**Day 5**	**Day 7**	**Day 9**	**Day 11**	**Day 17**
		Control					
1	1	38.9	39.9	38.9	38.3	38.7	38,1
2	3	39.0	39.5	39.9	38.5	39.5	38,2
3	7	39.0	39.5	40.0	38.3	38.7	38,4
4	8	39.4	39.5	38.4	38.3	38.9	39,1
5	9	39.0	39.5	40.0	38.0	38.8	39,0
6	10	39.2	39.3	39.7	38.3	38.6	38,2
7	13	38.7	39.5	38.7	38.1	38.4	39,0
The average value		39,0	39.5	39.4	38.3	38.8	38.6
		Experiment					
1	2	39.4	39.3	39.5	38.5	39.3	38,3
2	4	39.0	39.3	39.5	38.1	38.6	38.2
3	5	38.8	41.0	39.7	38.5	38.3	39.3
4	6	38.6	42.0	39.9	39.9	38.9	38.2
5	11	39.1	39.4	40.0	38.6	38.2	38.7
6	12	38.3	39.1	39.7	38.5	38.5	38.2
7	14	39.0	39.9	38.6	38.1	38.6	39.2
The average value		38,9	40.0	39.5	38.6	38.6	38.6

By the fifth day of the experiment, wound sizes in some animals from both groups increased by 0.5 to 1.0 cm compared to baseline measurements. On the seventh day, wound size reduction was observed in four animals from the control group (by 1.0 cm). In comparison, five animals in the experimental group exhibited a decrease ranging from 0.5 to 1.0 cm. By the ninth day, two rabbits in the control group showed an expansion of wound boundaries by 1.0 cm, whereas four rabbits exhibited a decrease of 0.5 to 1.0 cm; in one rabbit, the wound size remained unchanged **(Figure 1)**.



a) b)

c d)

Figure 1. Dynamics of infected wound healing in both groups. a) Experimental group, Day 3 of combined therapy; b) Experimental group, Day 30 of follow-up; c) Control group, Day 3 of saline solution treatment; d) Control group, Day 30 of follow-up

By the ninth day, three rabbits in the experimental group showed a reduction in wound size ranging from 0.5 to 2.0 cm, while in four rabbits, the wound dimensions remained unchanged compared to the seventh day. On the eleventh day, all rabbits in the control group exhibited a decrease in wound size of 3.0 to 4.0 cm. However, in five rabbits, a small amount of purulent exudate persisted, indicating a sluggish wound repair process. Additionally, two animals presented with moist wounds, and the surrounding soft tissues appeared hyperemic and edematous **(Table 2)**.

Table 2. Infected wound sizes

##	Animal number	Infected wound size, cm					
		Day 1	Day 5	Day 7	Day 9	Day 11	Day 17
				Control			
1	1	5.0	5.0	4.0	5.0	4.0	The scab. Pus under the scab when treatments
2	3	5.0	5.0	4.5	4.0	4.0	The scab. Pus under the scab when washing
3	7	4.5	5.0	5.0	4.0	3.5	The scab. During washing and treatment, a scanty purulent discharge
4	8	5.0	5.0	5.0	5.0	4.0	Healing under the scab
5	9	4.5	4.5	5.0	4.0	4.0	Healing under the scab
6	10	5.0	5.0	4.0	5.0	3.0	Healing under the scab
7	13	5.5	5.5	4.5	4.0	3.5	Healing under the scab
				Experiment			
1	2	4.5	5.0	4.5	3.0	Healing under the scab	Healing under the scab
2	4	4.5	4.5	5.0	3.0	Healing under the scab	Healing under the scab
3	5	5.0	5.0	4.5	4.5	3.0	Healing under the scab
4	6	5.0	5.0	4.0	4.0	Healing under the scab	Healing under the scab
5	11	4.5	4.5	4.0	4.0	2.0	Healing under the scab
6	12	3.5	4.5	3.5	3.0	3.0	Healing under the scab
7	14	5.5	5.5	5.0	5.0	3.5	Healing under the scab

By day 11, tissue regeneration in the experimental group was significantly more active and effective compared to the control group. In three rabbits, the wounds were nearly completely healed and covered by a thin, dry scab. Among the remaining four animals, wound sizes ranged from 2 to 3 cm. By day 17, three rabbits in the control group still exhibited purulent phenomena associated with scab formation, whereas all other animals had fully healed wounds. In the experimental group, all rabbits demonstrated complete wound closure, and no purulent discharge was observed (Blinov *et al*., 2022; García & Jaramillo, 2023; Ingle *et al*., 2023).

Microbiological analysis on day five revealed that a monoculture of *Staphylococcus epidermidis* was isolated from two animals in the control group. Additionally, *Klebsiella oxytoca* (a Gram-negative bacterium) was found in four animals, and *Staphylococcus aureus* (Gram-positive) was identified in three animals. In the experimental group, two animals exhibited a microbial association consisting of Escherichia coli, Staphylococcus aureus (10^6 CFU/ml), and *Klebsiella oxytoca* (10^6 CFU/ml). *Klebsiella oxytoca* was isolated from two rabbits, Staphylococcus aureus from two rabbits, and a combination of *Klebsiella pneumoniae, Streptococcus hemolyticus,* and *Escherichia coli* was found in one rabbit.

By day 14, no microflora was detected in the experimental group subjected to distant non-invasive electromagnetic therapy, indicating complete microbial clearance. In contrast, the microbial landscape in the control group shifted notably during the secondary phase of wound healing, with an overall decrease in bacterial load by 90%. In the control group, *Staphylococcus epidermidis* and *Klebsiella oxytoca* were each found in three animals, *Staphylococcus aureus* in one animal, and *Klebsiella pneumoniae* in one animal.

On day 30*, Staphylococcus epidermidis* was isolated from four animals in the control group, *while Klebsiella oxytoca was isolated from one animal. N*o microbial growth was observed in two animals. Notably, in the experimental group, 100% of animals exhibited no detectable microflora on the healed skin, and all wounds had fully closed. Furthermore, no visible scarring was observed in the areas of wound repair among the experimental animals, and normal fur growth was noted at these sites **(Table 3)**.

Table 3. Microbiological landscape of wounds treated with distant non-invasive electromagnetic therapy

##	Animal number	Wound content bacteriological examination, day	
		Day 5	Day 14
		Control	
1	2	*Staphylococcus epidermidis*, 10^4CFU/ml	*Klebsiella oxytoca,* 10^3CFU/ml
2	4	*Klebsiella oxytoca,* 10^6CFU/ml	*Klebsiella oxytoca,* 10^3 CFU/ml
3	5	*Staphylococcus aureus*, 10^5 CFU/ml	*Staphylococcus epidermidis,* 10^3 CFU/ml
4	6	*Klebsiella oxytoca,* 10^7CFU/ml	*Staphylococcus aureus,* 10^2 CFU/ml
5	11	S*taphylococcus epidermidis,* 10^5CFU/ml	*Klebsiella pneumoniae,* 10^3 CFU/ml
6	12	*Staphylococcus aureus,* 10^6 CFU/ml	*Staphylococcus epidermidi*s, 10^3 CFU/ml
7	14	*Klebsiella oxytoca,* 10^7CFU/ml	*Staphylococcus epidermidis,* 10^4 CFU/ml
		Experiment	
8	1	*Klebsiella oxytoca*, 10^7CFU/ml *E. Colli,* 10^7 CFU/ml	Microflora not detected
9	3	*Staphylococcus aureus,* 10^6 CFU/ml *Klebsiella oxytoca,* 10^6 CFU/ml	Microflora not detected
10	7	*Staphylococcus aureus,* 10^6 CFU/ml	Microflora not detected
11	8	*Staphylococcus aureus,* 10^6 CFU/ml	Microflora not detected
12	9	*Klebsiella pneumoniae,* 10^6 CFU/ml	Microflora not detected
13	10	*Staphylococcus aureus,* 10^6 CFU/ml	Microflora not detected
14	13	*Streptococcus haemolyticus,* 10^6 CFU/ml	Microflora not detected

Prior to the experiment, the average white blood cell count in rabbits was within normal physiological ranges. On day 5, leukocyte counts in the control group exceeded the upper physiological limit by 3.0%, whereas the experimental group exhibited an increase of 68%. By day 14, leukocyte levels in both groups returned to within the physiological norm, with the control group displaying values lower than the initial baseline.

Platelet counts showed a 1% increase in the control group and a 32% increase in the experimental group by day 5. By day 14, platelet counts in the control group had decreased by 57% compared to baseline, while the experimental group demonstrated a 71% increase in platelet activity relative to initial values following distant non-invasive electromagnetic therapy **(Table 4)**.

Table 4. Average values of hematological parameters of rabbits in both groups

##	Indicator	Group	Day 1	Day 5	Day 14
1	Leukocytes, 10^9/l	Control	6.1±0.1	10.2±0.2	5.5±0.1

		Experiment	5.0±0.1	16.6±0.2	7.3±0.1
2	Neutrophils, %	Control	49.8±0.6	76±1	61.7±0.9
		Experiment	55.6±0.7	81.7±0.2	68.3±0.4
3	Lymphocytes, %	Control	50±0.6	24±1	37.6±0.9
		Experiment	44.4±0.7	18.3±0.4	31.7±0.4
4	Trombocytes,10^9/l	Control	497.6±11.7	501±15	284.9±7.1
		Experiment	309±2.7	409±14	527.7±10.7

Elevated body temperature, or hyperthermia, is a hallmark of the initial stage of the wound healing process (the inflammatory reaction phase) and is associated with the release of prostaglandins and prostacyclins into the bloodstream, which influence the thermoregulatory center. Our preclinical trials demonstrated that exposure to electromagnetic fields (EMF) accelerated this initial phase, facilitating a rapid progression to scar proliferation and tissue remodeling (Yuan *et al*., 2018; Ploskonos *et al*., 2022; Kızılcı *et al*., 2024). Notably, the experimental group exhibited pronounced leukocytosis, primarily due to increased neutrophil counts. This response enhanced the elimination of pathogens and debris from cellular and tissue destruction, leading to a well-defined inflammatory barrier. The subsequent phases of wound healing in the experimental group were also shortened in comparison to the control group, attributed to heightened fibroblast activity. Constriction processes, intensified by EMF therapy, were marked by a synergistic progression of epithelialization and the maturation of granulation tissue through fibrinogenesis (Betskii *et al*., 2000; Subbotina *et al*., 2006; Pokorný *et al*., 2008; Heimes *et al*., 2022).

The effectiveness and regulation of "respiratory stress" processes were evidenced by the absence of purulent discharge in the experimental group by day 11. In contrast, suppuration persisted in the control group beyond this time-point. The increased rate of wound repair associated with non-invasive EMF therapy can also be linked to the aseptic conditions maintained during the regenerative processes of the second healing phase (Wang, 2019; Dijksteel *et al*., 2020; Wang *et al*., 2021; AlHumaidi *et al*., 2022; Guo *et al*., 2022; Li *et al*., 2022; Ma *et al*., 2022; Pang *et al*., 2023).

By day 14 and thereafter, no microbial growth was detected on the wound surfaces in the experimental group. In contrast, the control group continued to exhibit a diverse population of both gram-positive and gram-negative bacteria, which impeded healing (Chen *et al*., 2016, 2017; Zhang *et al*., 2017; Reshetnikov & Barashkova, 2022). Importantly, wounds in the experimental group healed 25% faster than those in controls.

A significant increase in platelet count was observed at all stages of recovery in the experimental group. It is well established that, beyond their role in hemostasis, platelets contain up to 100 biologically active granules per cell (Fiodorova *et al*., 2022; Li *et al*., 2024; Wang *et al*., 2024). These granules store a variety of substances, including growth factors, histamine, serotonin, elastase, collagenase, protein, and amyloid precursor, which collectively stimulate angiogenesis in developing connective tissue. Furthermore, these molecules possess chemotactic properties for lymphocytes, macrophages, and neutrophils, thereby enhancing local immune responses (Schlesinger, 2018; Scopelliti *et al*., 2022; Wilhelmy *et al*., 2022; Burnouf *et al*., 2023; Macrì *et al*., 2023).

Conclusion

In conclusion, the findings of this preclinical study demonstrate that remote non-invasive electromagnetic therapy significantly accelerates the healing process of experimentally infected wounds in a rabbit model. The application of low-intensity, non-ionizing, pulsed electromagnetic fields (PEMF) served as a highly effective adjunct to standard wound management, markedly improving regenerative outcomes.

The experimental group, which received PEMF therapy, exhibited a profoundly enhanced and accelerated inflammatory response phase, a critical first step in wound healing. This was evidenced by a pronounced leukocytosis, with white blood cell counts increasing by 68% to $16.6 \pm 0.2 \times 10^9$/l on day 5, compared to a mere 3.0% increase in the control group. This robust cellular response, driven predominantly by neutrophils, facilitated more efficient pathogen clearance and debridement of necrotic tissue. Consequently, the progression to subsequent proliferative and remodeling phases was markedly shortened. A pivotal finding was the complete microbial clearance achieved in the experimental group by day 14, a stark contrast to the control group, which continued to harbor a diverse population of Gram-positive and Gram-negative bacteria such as *Staphylococcus epidermidis* and *Klebsiella oxytoca*.

Furthermore, the therapy stimulated crucial processes in tissue regeneration. A significant 71% increase in platelet activity was observed in the experimental group by day 14, highlighting a key systemic benefit. Beyond their role in hemostasis, platelets released a plethora of biologically active granules that promoted angiogenesis, enhanced local immune responses, and stimulated fibroblast activity. This resulted in more effective constriction, epithelialization, and maturation of granulation tissue. Clinically, this translated to a complete absence of purulent discharge in all experimental animals by day 11 and full wound closure without visible scarring by the end of the 30-day follow-up period. Overall, the wounds in the experimental group healed 25% faster than those in the control group.

These results strongly indicate that non-invasive electromagnetic therapy not only provides a potent local effect on the wound bed but also exerts a significant systemic immunostimulatory action. The therapy presents a promising, non-pharmacological modality to complement classical methods for treating infected wounds, potentially reducing healing times and improving cosmetic outcomes. However, the precise molecular and cellular mechanisms underlying this accelerated healing warrant further detailed investigation to harness its therapeutic potential fully.

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Ethics statement: The study protocol was approved by the Bioethics Committee of the Far Eastern State Agrarian University, Russian Federation (Protocol No. 7, dated 2024/04/14). All procedures adhered to the"National General Ethical Principles of Animal Experimentation" and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (March 18, 1986).

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