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Fabrication of CeO_2 nanoparticles embedded in polysaccharide hydrogel and their application in skin wound healing

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Nanocrystalline cerium oxide (CeO_2) is considered as one of the most promising inorganic materials for biomedical purposes. The unique redox-activity, high biocompatibility and low toxicity of CeO_2 nanoparticles open great prospects in their biomedical usage as a therapeutic agent, including acceleration of skin regeneration processes after injuries of various etiologies. As part of this work, a hydrogel based on natural polysaccharides modified with CeO_2 nanoparticles was synthesized and its therapeutic efficacy in the treatment of planar full thickness and linear skin wounds in rats was shown. Basing on wound surface area measurements, results of skin wounds tensiometry and histological analysis it was found that polysaccharide hydrogel significantly reduces planar and linear wound healing times. Polysaccharide hydrogel modified with CeO_2 nanoparticles facilitates rapid reduction of wound defect area and the scar formation with complete tissue regeneration in the wound area. Additionally, composite hydrogels reduce the manifestations of non-specific signs of inflammation and intoxication. Thus, polysaccharide hydrogel modified with CeO_2 nanoparticles can be regarded as an effective wound healing substance in the therapy of skin injuries of various etiologies.

Keywords: wound healing, hydrogel, cerium oxide nanoparticles, cutaneous application, regeneration, cell proliferation.

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1. Introduction

One of the most promising nanomaterials for biomedical applications is nanocrystalline cerium dioxide (i.e., CeO_2 nanoparticles). The unique redox activity of CeO_2 nanoparticles provides its specific activity in a biological microenvironment [1–4] that drastically distinguishes cerium from the other elements of the lanthanides family. CeO_2 nanoparticles are able to inactivate a wide range of reactive oxygen species (ROS), even at nanomolar concentrations, effectively preventing intracellular oxidative stress [5, 6]. The physico-chemical and biological behavior of CeO_2 nanoparticles in the cell is dictated by many factors. To explain the bioactivity of CeO_2 nanoparticles, most authors extrapolate existing data on their activity in catalytic processes (cracking, exhaust gas after burning, organic oxidation) to biological processes, describing CeO_2 nanoparticles as a "biological catalyst", nanozyme [7, 8]. It is generally accepted that the unique biological activity of CeO_2 nanoparticles is affected by several factors, including the shape, size, crystallinity and surface charge of the particles [9]. The above characteristics depend on the synthesis conditions, the nature of the precursors and surfactants, etc [10].

Earlier, we showed the possibility of using CeO_2 nanoparticles to accelerate the proliferation of human mesenchymal stem cells (MSC) isolated from dental pulp and human Wartan jelly, as well as mouse embryonic stem cell culture [11–13]. CeO_2 nanoparticles inhibited intracellular oxidative stress directly by inactivation of ROS and indirectly via modulation of gene expression, which provided natural and microenvironment for cell proliferation and migration. Additionally, it has been shown that polylactide scaffolds modified with CeO_2 nanoparticles can be used as unique material for effective growth and proliferation of human mesenchymal stem cells [14]. Moreover, CeO_2 nanoparticles are capable of enhancing the viability of neuronal cell cultures, including astrocytes, neurons, oligodendrocytes and microglial cells [15, 16]. CeO_2 nanoparticles application into neuronal cell culture leads to prolongation of their life span up to 280 days. Thus, CeO_2 nanoparticles are able to stimulate the growth and proliferation of various types of cell cultures *in vitro* [17]. Meanwhile, all previous studies demonstrate its efficacy in various models only, skipping over the detailed research of the molecular mechanisms of its activity.

In this work, a polysaccharide hydrogel based on carboxymethyl cellulose, chitosan, fucoidan and carrageenan modified with citrate-stabilized CeO_2 nanoparticles was synthesized and its therapeutic efficacy was shown in the treatment of model planar and linear skin wounds in rats.

2. Materials and methods

2.1. Laboratory animals. Animal care and maintenance

Experimental procedures were performed on Wistar white rats. Rats weighing 180 – 190 g at the age of 12 – 13 weeks were used. Animal maintenance was carried out in accordance with the rules adopted by the European Convention for the Protection of Vertebrate Animals (Strasbourg, 1986). The animals were fed twice a day. Animal maintenance was in accordance with the rules of Good Laboratory Practice (GLP) and the Order of the Ministry of Health of the Russian Federation No. 199n "Rules of Good Laboratory Practice". Animals had unlimited access to water using special drinking bottles for rodents. Preliminary bacteriological analysis and sanitary chemical examination of water was performed. There was no contamination of the bedding, feed and water that could affect the results of the study. During the study each animal was examined daily. The examination included an assessment of overall behavior and systemic condition of animals. When applying test preparations, the examination was carried out approximately 2 hours after the application. Visual characteristics were recorded by photographing the wound on the 2nd, 7th, 14th and 28th days. Wound sizes were recorded by metric methods.

2.2. Vital signs monitoring in laboratory animals

Animal weighing was carried out immediately prior to wound simulation on the 2nd, 7th days, and then once a week to control body weight dynamics and immediately prior to euthanasia to calculate the percentage of organ weight to body weight. Blood samples were collected from animals before the start of the experiment, on the 2nd day after the wound simulation (puncture of the tail vein) and immediately before euthanasia. On the day before blood sampling feed-troughs with food were withdrawn from rat cages. The next day, the animals were sacrificed in a CO_2 chamber and blood samples were collected to analyze the following parameters: hemoglobin, erythrocyte count, leukocyte count, leukogram, platelets, lymphocytes, monocytes, neutrophils. Finally, pathomorphological studies were also performed. The pathomorphological study included necropsy, macroscopic examination, weighing of internal organs, and histological examination of the wound surface of the skin.

2.3. Simulation of skin integument injury

2.3.1. Formation of a planar full-thickness wound. A day prior to the experiment, hair-coat of rats was trimmed at the site of the intended wound infliction. Animals were immobilized by means of ether inhalation anesthesia, a circular flap of skin of 60.0 ± 1.0 mm^2 was cut off with rounded-end surgical scissors from cervico-occipital region using a stencil. Wounds remained open until the end of the experiment. Levomecol ointment was used as a control medication. Animals were divided into 4 groups of 6 test subjects in each. The first group was intact without wound infliction. The second one was a control group without drug application onto the wound, with spontaneous wound healing. In the third group, Levomecol ointment was applied onto the wound. In the fourth group, a CeO_2 containing hydrogel was applied onto the wound. Immediately after the operation and then daily for 14 days experimental groups of animals were treated with hydrogels in an amount of 0.2 g. The hydrogel was preheated in a water bath to 38 – 40 °C and then applied with a glass rod onto the wound surface. Thereafter, the animals were placed for 2 hours into special individual plastic containers to restrict locomotor activity and to prevent gel licking. After releasing of rats from the containers, the excess of the gel was removed from the skin with filter paper. The change in wound surface area was recorded once a day by measuring the wound diameter using the vernier caliper (length = 125 mm, accuracy = 0.01 mm). The wound surface area was then calculated:

$$S = \frac{\pi \times D^2}{4},$$

where S is the wound area, D is the wound diameter, $\pi = 3.14$.

2.4. Formation of a linear wound

A day prior to the experiment, hair-coat of rats was trimmed at the dorso-lumbar area. The animals were anesthetized with diethyl ether by inhalation. A linear full-thickness skin wound 5.0 cm long was created in the skin of the depilated rat's back applying the stencil using a surgical scalpel, the wound edges were brought together and sutures were applied at an equal distance. Sutures were applied so that the epithelium of the lateral edges of the wound did not come into contact, and the epithelization took place from the terminal edges of the wound. The suture material used was sterilized silk filament #0, which was removed on the 5th day of the experiment. Wounds remained open

until the end of the experiment. During the experiment the body temperature of the animals was measured. Blood samples were collected prior to the experiment, on the 2nd and 7th days after linear wound formation, morphological parameters of blood and leukocyte formula were determined. The total duration of observations was 7 days. The efficacy of pharmaceutical agents used was evaluated basing on the observations of linear wounds healing dynamics, the performance status of the animals was assessed as well as the presence or absence of an inflammatory process. On the 7th day after wound formation animals were euthanized by sacrificing in a CO_2 chamber followed by the withdrawal of wound area full-thickness skin flap of 2 cm $\times$ 3 cm. According to the recommendations of the guide to preclinical trials of pharmaceutical agents the scar-breaking strength was determined using wound tensiometer [18].

2.5. Polysaccharide hydrogel synthesis and modification

The hydrogels were made from citrate-stabilized cerium oxide nanoparticles prepared according to [19], pectin, fucoidan, sodium alginate, water-soluble derivatives of cellulose (carboxymethyl cellulose) and purified water. Briefly, 1.0 g fucoidan, 1.0 g sodium alginate, 8.0 g carboxymethyl cellulose were dissolved in 100 ml distilled water. The solution was mixed at 25 °C and then left at 4 °C for 96 h until the formation of a homogeneous gel. Then 100 μl of the CeO_2 sol (CeO_2 concentration in the sol was 10^{-2} M) was added and the mixture was vigorously homogenized. Before application, the gel was sterilized in an autoclave.

2.6. Statistical data treatment

Statistical treatment of the results was performed using STATISTICA 8.0 software to evaluate the Student's t-criterion. Data are presented as the sample mean M, the standard error of the mean m, and the achieved level of significance p. Accepted minimum significance level of differences was taken as $p \leq 0.05$. In some cases, especially when there is a natural trend of indicators (e.g. animal body weight gain over the observation period, etc.), the methods of dispersion analysis were used.

3. Results

Data obtained indicate that CeO_2-containing hydrogel is well tolerated by the animals, has no irritating effect, does not cause hyperemia and skin edema, and does not affect the level of physical activity in test animals. During the observation period, no cases of death, wound abscess or complicated course of wound process were registered. The gel could easily be applied onto the wounds, adsorbing wound excretions, not drying wound bottom, and maintaining a wet environment in the wound. The results of comparative assessment of wound healing activity of hydrogel and Levomecol ointment on a model planar full-thickness wound are presented in Table 1.

Analysis of the experimental study results showed that throughout the test period a gradual decrease in the wound surface area at all stages of the wound process was observed in the control group. In the group of animals treated with Levomecol ointment, the wound area is reduced on the 9th day by 16.38 % relative to the control ($p < 0.05$).

In the experimental group, where wounds were treated with hydrogel, there was an acceleration in wound healing by 3 days; on the 8th and 11th days wound surface area decreased by 40.51 % and 39.99 %, respectively, relative to the control ($p < 0.05$).

The maximum effect is observed in a group of animals treated with hydrogel at all stages of wound healing process (Fig. 1).

In histological sections of the skin of the intact group of animals, the epidermis is characterized by a classical histological structure (Fig. 2(a1)). Cells of epithelial tissue of ectodermal origin of basal layer have a regular cubic shape and are arranged in line abreast, there are very few mitotic cells. In the depth of the spinous layer, the cells are cubic, while near the next layer, they become flatter (Fig. 2(a2)). The corneum layer is formed by several layers of corneal cells comprising keratin. The papillary layer is formed by a loose fibrous connective tissue consisting of collagen, elastin and reticulin fibers randomly interconnected with each other (Fig. 2(a3)). Up to seven hair follicles fall within one field of vision, forming complexes together with sebaceous glands (Fig. 2(a3)). In histological sections, the identified dermal papillae are very few, being randomly arranged. Fibroblasts are located mainly in subepidermal layers. The vessels are few, small and well evident.

In the control group of animals, a thin scar is formed with complete recovery of the epidermis in the wound region. On day 14, the epidermis is significantly enlarged, cells of all skin layers have a variety of sizes and shapes (Fig. 2(b1)). Cellular vacuolization is observed in the spinous layer of the epidermis (Fig. 2(b2)). The papillary layer of dermis is poorly resolved (Fig. 2(b1, b2)). Vascular blood filling is uneven, some blood vessels are collapsed, in the form of "cellular chorda", the other ones are moderately filled with blood together with weak leukocyte infiltration. Epithelium growth occurs across the surface of the granulation tissue and loose connective tissue that is formed during dermal regeneration. Granulation tissue cells proliferate around the blood vessels in the form of small clusters. Proliferative processes are dominated in regenerating tissue resulting in formation of focal cell infiltrates with the proliferation of

TABLE 1. Wound healing activity of ceria-containing hydrogel and levomecol ointment on planar full thickness skin wound model in rats

Day	**Surface area of planar full-thickness wound, cm^2**		
	Control	**Levomecol ointment**	**CeO_2 Hydrogel**
0	0.65 ± 0.03	0.62 ± 0.03	0.63 ± 0.05
1	0.58 ± 0.07	0.57 ± 0.05	0.58 ± 0.08
2	0.58 ± 0.07	0.58 ± 0.04	0.56 ± 0.08
3	0.56 ± 0.07	0.54 ± 0.06	0.48 ± 0.03*
4	0.57 ± 0.05	0.54 ± 0.07	0.43 ± 0.05* **
5	0.56 ± 0.05	0.54 ± 0.07	0.36 ± 0.03* **
6	0.53 ± 0.07	0.52 ± 0.06	0.37 ± 0.04* **
7	0.49 ± 0.07	0.47 ± 0.06	0.33 ± 0.03* **
8	0.48 ± 0.07	0.43 ± 0.05	0.21 ± 0.06* **
9	0.40 ± 0.06	0.28 ± 0.08*	0.16 ± 0.08* **
10	0.34 ± 0.07	0.21 ± 0.04*	0.10 ± 0.05* **
11	0.26 ± 0.04	0.17 ± 0.04*	0.01 ± 0.01* **
12	0.14 ± 0.04	0.07 ± 0.05*	0
13	0.09 ± 0.07	0.03 ± 0.04*	0
14	0.09 ± 0.07	0.01 ± 0.05*	0

* – significant difference of parameters from control ($p < 0.05$)
** – significant difference of parameters from reference preparation (at $p < 0.05$)

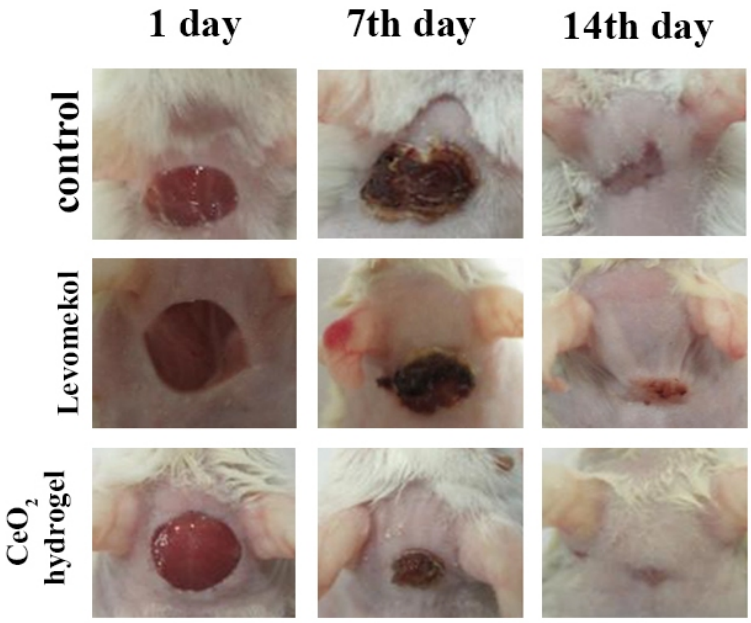

FIG. 1. Appearance of planar full-thickness wound in rats

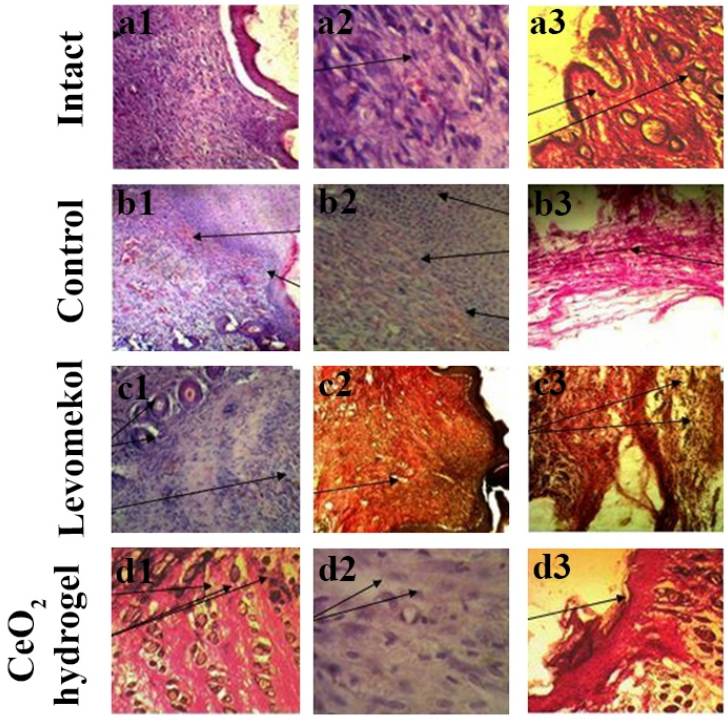

FIG. 2. Histological analysis of the epidermis of the skin of rats treated with various substances

granulation tissue and fibrous connective tissue (Fig. 2(b3)). Strands of collagen fibers are located in parallel to skin surface. Neither hair follicles nor sebaceous glands were found.

In the group of animals treated with Levomecol ointment, the epidermis is moderately or significantly thickened, elongated cell nuclei are horizontally oriented (Fig. 2(c1)). In the underlying dermis, macrofocal profound homogenization is registered, moderate basophilia of dermal collagen fibers could be observed, the blood vessels of the dermis are congested with blood (Fig. 2(c2)). In adjacent skin, there is microfocal weakly expressed epidermal cell swelling in the form of translucent perinuclear spaces. Newly formed blood vessels of different diameters with expanded lumen could be distinguished, they are congested with blood with erythrostases, diapedetic microhemorrhages (Fig. 2(c3)), the formation of sporadic hair follicles is observed (Fig. 2(c3)), and sebaceous glands were not found.

In the group of animals where the wounds were treated with CeO_2-containing hydrogel, scar formation occurs with complete recovery of the epidermis in the wound region. The scarring zone is visually smaller in area as compared to control groups. There is a proliferation of granulation tissue that fills the entire wound defect region and has a typical structure (Fig. 2(d1)). Among cells of developing tissue, cells of circulatory bed as well as of tissular origin were revealed, including fibroblasts located around small capillaries (Fig. 2(d2)). Epithelial sheets of regenerating tissue are formed from the edges of wound defect as two arrays towards each other and cover the wound. In the animals, on day 14 after wound infliction, the epithelium is almost completely restored in the region of skin injury. The epithelial cells of the basal layer proliferate deep into the dermis, forming intergrowths. As the wound heals, a cornea layer of the epidermis is formed (Fig. 2(d3)). Collagenization processes occur at the site of the tissue defect. Mature connective tissue is formed, and the generation of hair follicles and sebaceous glands take place inside it (Fig. 2(d1)).

Thus, the use of the hydrogel reduces inflammatory changes, activates macrophagal response, restores impaired intercellular interactions, enhances angiogenesis, proliferation and differentiation of fibroblasts, synthesis and secretion of collagen, fibrillogenesis processes, maturation, and remodeling of granulation tissue and its epithelization.

Our studies indicate that treatment with cerium-containing hydrogel accelerates regeneration of the planar full-thickness wound of rat skin that is expressed in the formation of hair follicles and sebaceous glands in the wound area, as well as in remarkable reduction in the scarring area, without causing reorganization of the scarring structure.

Animals were observed within 14 days after wound simulation. Observation of the overall condition and behavior, weighing, monitoring of feed and water consumption were carried out daily. After 14 days, all animals in the test groups were euthanized in a CO_2 chamber and dissected. Hematological studies, macroscopic description and the

evaluation of mass coefficients of internal organs, histological studies were carried out. The results of animal body weight measurements are shown in Table 2.

Table 2 shows that the body weight of animals is reduced after injury. Subsequently, the weight growth rates of control animals and Levomecol-treated animals are lower than the results of the intact group. When the hydrogel is used, these body weight values are not significantly different from intact control. The revealed changes are not statistically significant. No significant differences in body weight dynamics were found in the groups of animals treated with Levomecol and CeO_2-containing hydrogel.

2 hours after drug epicutaneous application, rats were relocated into exchange cages for 24 hours to evaluate water and food consumption. The results are shown in Tables 3, 4.

TABLE 2. Body weight dynamics of male white rats (g, $M \pm m$) during the treatment of planar full-thickness skin wound

	Test group			
	Intact animals	**Control**	**Levomecol ointment**	**CeO_2 Hydrogel**
Initially	189.0 ± 4.8	187.2 ± 2.1	185.1 ± 4.4	180.7 ± 3.0
2-nd day	191.3 ± 3.6	180.2 ± 3.1	182.4 ± 4.7	178.0 ± 3.8
7-th day	196.2 ± 4.3	182.7 ± 2.9	187.4 ± 5.5	195.7 ± 3.3
14-th day	204.4 ± 4.3	188.4 ± 3.8	192.6 ± 5.1	201.5 ± 2.8

TABLE 3. Water consumption dynamics (ml/day) by rats during the treatment of planar full-thickness skin wound ($M \pm m$)

	Test group			
	Intact animals	**Control**	**Levomecol ointment**	**CeO_2 Hydrogel**
Initially	20.2 ± 1.5	19.0 ± 0.3	19.8 ± 1.3	21.7 ± 0.5
2-nd da y	19.0 ± 0.4	14.1 ± 1.5*	15.2 ± 0.9*	13.7 ± 0.2*
7-th day	19.4 ± 1.2	19.4 ± 0.3	21.1 ± 1.0	17.9 ± 0.7
14-th day	19.7 ± 0.9	18.9 ± 0.6	18.3 ± 0.3	18.5 ± 0.2

* – Significant differences from initial values and results of the intact group ($p < 0.05$)

TABLE 4. Food consumption dynamics (g/day) by rats during the treatment of planar full-thickness skin wound ($M \pm m$)

	Test group			
	Intact animals	**Control**	**Levomecol ointment**	**CeO_2 Hydrogel**
Initially	19.2 ± 2.2	19.9 ± 0.8	20.1 ± 2.3	19.6 ± 2.3
2-nd day	20.9 ± 2.8	10.2 ± 3.0*	11.8 ± 2.5*	10.4 ± 3.5*
7-th day	21.4 ± 1.3	18.9 ± 2.1	21.5 ± 1.4	22.1 ± 1.2
14-th day	20.2 ± 2.4	23.7 ± 1.7	21.7 ± 1.4	20.9 ± 1.8

* – Significant differences from initial values and results of the intact group ($p < 0.05$)

As it could be seen from Tables 3 and 4, in the control group and the groups of animals treated with the tested preparations, on the 2nd day after administration the amount of water consumed is statistically reduced and the food consumption is reduced considerably in comparison with the initial level and with the intact group. Subsequently, the results of the evaluation of water and food consumption in the control and intact groups did not vary. There were no significant differences in these values in the groups of animals receiving the tested preparations.

As a non-specific sign of inflammation during the treatment of planar full-thickness skin wound using Levomecol ointment and CeO_2-containing hydrogel, the body temperature of rats was determined. Rectal temperatures were measured using an electronical medical thermometer (permissible intrinsic error for the range of measured temperatures ± 1 %) 2 hours after drug epicutaneous application. The data obtained are shown in Table 5.

TABLE 5. Rectal temperature of rats during the treatment of planar full-thickness skin wound ($^\circ$C, $M \pm m$)

	Test group			
	Intact animals	**Control**	**Levomecol ointment**	**CeO_2 Hydrogel**
Initially	37.6 ± 0.2	37.3 ± 0.3	37.3 ± 0.2	36.9 ± 0.4
2-nd day	36.8 ± 0.3	38.9 ± 0.4*	38.9 ± 0.4*	38.1 ± 0.3*
7-th day	36.8 ± 0.2	38.1 ± 0.3*	38.0 ± 0.2*	37.1 ± 0.6
14-th day	36.8 ± 0.3	38.0 ± 0.3*	37.6 ± 0.3	37.3 ± 0.4

* – Significant differences from initial values and results of the intact group ($p < 0.05$)

It was found that on the 2nd day after planar full-thickness wound infliction the body temperature in animals of the control group is increased relative to the initial values. On the 7th and 14th days of observation, body temperature remains elevated. In the Levomecol ointment treated group, the results are not significantly different from the control ($p < 0.05$). In the CeO_2-containing hydrogel treated group, a reduction in rectal temperature to the initial level was observed on day 7 after the wound infliction.

Hematological studies were carried out prior to wound simulation and after 2 and 14 days of daily drug application in all groups. Blood samples were collected by puncture of the tail vein, and at the end of the experiment – after euthanasia of the animals.

The data obtained are shown in Tables 6, 7.

On the 2nd and 14th day, the control group showed an increase in ESR by 166.3 and 77.2 % as well as in leukocytes by 43.6 and 21.0 %, respectively, compared to the initial values ($p < 0.05$) that indicates an inflammatory process in animals. Levomecol ointment showed a significant decrease in ESR and number of leukocytes on day 2. In the group of animals treated with CeO_2-containing hydrogel, on day 2 there was a significant decrease in ESR and a decrease in leukocytosis relative to control, on day 14 there was a decrease in ESR and the number of leukocytes returns to the initial level.

No statistically significant differences were found in the values of other indicators

The ratio of leukocyte forms (Table 7) in animals in the intact group prior to the start of the experiment corresponds to the normal values typical to the healthy rats. Initial values in the control and all test groups are within normal limits and indicate the absence of inflammatory processes in animals prior to the start of the experiment.

In the control group, on the 2nd and 14th days after the wound simulation there was a decrease in the percentage of lymphocytes by 5.0 and 3.3 %, respectively, compared to initial values. On day 2, an increase in the number of segmentonuclear and banded neutrophils compared to intact group and initial values was observed, indicating the presence of neutrophilic leukocytosis and an inflammatory process.

Application of Levomecol ointment onto a linear wound for 14 days resulted in a slight increase in the relative number of lymphocytes compared to the control. The number of segmentonuclear and banded neutrophils did not differ significantly from the control, which indicated the inability of Levomecol ointment to prevent the leucogram deviation. When using CeO_2-containing hydrogel, on day 14 there was an increase in lymphocyte count compared to the data for the control group, as well as a decrease in the number of segmentonuclear and banded neutrophils compared to the control. These values did not differ statistically from the initial ones. This indicates the ability of CeO_2-containing hydrogel to reduce the severity of the inflammatory process. No statistically significant changes in other leucogram parameters were found. At the end of the experiment, all the animals of control and test groups, as well as intact animals, were subjected to pathomorphological studies. Animals were withdrawn from the experiment

TABLE 6. The effect of drug epicutaneous application on peripheral blood morphological composition of white rats during the treatment of planar full-thickness wound ($M \pm m$)

	Test group			
	Intact animals	**Control**	**Levomecol ointment**	**CeO_2 Hydrogel**
Hemoglobin, g/dl				
Initially	13.9 ± 0.2	13.2 ± 0.1	13.1 ± 0.2	14.7 ± 0.1
2-nd day	12.7 ± 0.1	12.2 ± 0.3	12.2 ± 0.4	12.8 ± 0.4
14-th day	13.8 ± 0.2	12.7 ± 0.2	12.4 ± 0.3	13.7 ± 0.2
Hematocrit, %				
Initially	53.0 ± 2.6	51.4 ± 1.5	53.8 ± 1.0	51.9 ± 2.0
2-nd day	49.1 ± 1.3	58.7 ± 2.6	48.0 ± 1.5	49.4 ± 1.5
14-th day	50.7 ± 2.6	54.8 ± 1.3	52.3 ± 1.6	51.7 ± 2.1
Erythrocytes, $\times 10^{12}$/l				
Initially	6.6 ± 0.2	6.6 ± 0.2	7.2 ± 0.2	7.3 ± 0.3
2-nd day	6.9 ± 0.1	7.3 ± 0.1	6.4 ± 0.3	6.5 ± 0.2
14-th day	7.2 ± 0.1	6.4 ± 0.2	6.8 ± 0.3	6.9 ± 0.2
Blood colour index, pg				
Initially	18.9 ± 0.4	17.5 ± 0.4	17.9 ± 0.1	17.9 ± 0.4
2-nd day	17.9 ± 0.5	18.2 ± 0.6	18.6 ± 0.7	18.5 ± 0.5
14-th day	18.7 ± 0.6	17.5 ± 0.5	18.0 ± 0.4	17.3 ± 0.8
Leucocytes, $\times 10^9$/l				
Initially	9.2 ± 0.2	9.5 ± 0.4	10.1 ± 0.2	10.2 ± 0.3
2-nd day	10.3 ± 0.3	13.6 ± 0.2*	11.9 ± 0.5*	11.2 ± 0.4*
14-th day	9.7 ± 0.4	11.5 ± 0.7*	10.3 ± 0.4	10.3 ± 0.3
Thrombocytes, $\times 10^9$/l				
Initially	759 ± 47	721 ± 30	752 ± 75	725 ± 44
2-nd day	770 ± 34	748 ± 40	736 ± 38	715 ± 26
14-th day	783 ± 31	783 ± 70	749 ± 21	785 ± 34
ESR, mm/h				
Initially	4.5 ± 0.2	4.8 ± 0.4	4.5 ± 0.1	4.2 ± 0.1
2-nd day	4.0 ± 0.3	12.7 ± 1.2*	9.4 ± 2.6* **	7.9 ± 1.2* **
14-th day	5.2 ± 0.2	8.5 ± 1.1*	4.8 ± 3.7**	4.9 ± 3.6**

* – Significant difference from initial values (at $p < 0.05$)
** – Significant difference from the results of the control group (at $p < 0.05$)

TABLE 7. The effect of drug epicutaneous application on peripheral blood leucogram of white rats during the treatment of planar full-thickness wound ($M \pm m$)

	Test group			
	Intact animals	**Control**	**Levomecol ointment**	**CeO_2 Hydrogel**
Banded neutrophils, %				
Initially	2.4 ± 0.2	2.1 ± 0.1	2.2 ± 0.2	2.3 ± 0.2
2-nd day	2.2 ± 0.1	3.7 ± 0.4*	3.4 ± 0.2*	3.1 ± 0.1*
14-th day	2.4 ± 0.1	3.0 ± 0.2*	3.1 ± 0.1*	2.2 ± 0.1
Segmentonuclear neutrophils, %				
Initially	35.4 ± 1.0	35.0 ± 0.6	32.5 ± 1.1	35.6 ± 0.8
2-nd day	34.1 ± 0.6	41.4 ± 0.6*	39.5 ± 0.9*	40.6 ± 1.1*
14-th day	33.4 ± 0.4	40.9 ± 0.4*	36.1 ± 0.3**	31.5 ± 0.8**
Basophils, %				
Initially	0	0	0	0
2-nd day	0	0	0	0
14-th day	0	0	0	0
Eosinophils, %				
Initially	4.4 ± 0.1	2.6 ± 0.1	3.0 ± 0.2	2.9 ± 0.1
2-nd day	3.9 ± 0.1	2.7 ± 0.4	3.8 ± 0.2	3.3 ± 0.1
14-th day	4.6 ± 0.1	4.7 ± 0.1	3.8 ± 0.2	4.0 ± 0.2
Monocytes, %				
Initially	5.3 ± 0.1	5.1 ± 0.2	6.3 ± 0.2	5.7 ± 0.2
2-nd day	5.5 ± 0.4	5.9 ± 0.7	5.2 ± 0.1	6.8 ± 0.4
14-th day	5.7 ± 0.1	5.4 ± 0.4	5.4 ± 0.1	5.6 ± 0.3
Lymphocytes, %				
Initially	52.4 ± 1.1	57.3 ± 1.1	56.1 ± 0.7	53.3 ± 1.2
2-nd day	54.3 ± 0.7	46.4 ± 1.2*	48.1 ± 0.3	46.2 ± 0.8*
14-th day	53.9 ± 0.7	46.1 ± 0.5*	51.4 ± 0.7*	56.6 ± 0.5**
Plasmacytes, %				
Initially	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
2-nd day	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
14-th day	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1

* – Significant difference from initial values (at $p < 0.05$)
** – Significant difference from the results of the control group (at $p < 0.05$)

by sacrificing in a CO_2 chamber. Pathomorphological study included necropsy, macroscopic examination, internal organ weighing, and histological examination of the wound skin.

Table 8 shows the mass coefficients of organs of white rats from all experimental groups. It has been found that thymus mass coefficients in all experimental groups are significantly lower than the results of the intact group. The greatest differences with intact animals have been revealed in the case of Levomecol ointment use. The use of CeO_2-containing hydrogel partially normalizes these values, but the difference with control is unreliable.

TABLE 8. Mass coefficients of rat's internal organs during the treatment of planar full-thickness skin wound ($M \pm m$)

Organ	**Test group**			
	Intact animals	**Control**	**Levomecol ointment**	**CeO_2 Hydrogel**
Heart	4.2 ± 0.2	3.1 ± 0.1*	3.2 ± 0.3*	3.4 ± 0.3*
Lungs with a trachea	6.8 ± 0.2	6.9 ± 0.3	7.1 ± 0.2	7.0 ± 0.4
Timus	1.30 ± 0.12	1.03 ± 0.06*	1.01 ± 0.21*	1.18 ± 0.09*
Liver	36.0 ± 1.4	31.0 ± 1.4	36.3 ± 2.0	36.5 ± 2.5
Spleen	4.7 ± 0.2	4.2 ± 0.3	4.3 ± 0.2	4.5 ± 0.4
Kidney (left)	6.1 ± 0.3	6.0 ± 0.5	5.9 ± 0.5	5.9 ± 0.2
Adrenal gland (left)	0.11 ± 0.01	0.10 ± 0.01	0.09 ± 0.02	0.09 ± 0.01
Brain	7.5 ± 0.2	7.7 ± 0.2	7.6 ± 0.1	7.8 ± 0.1
Testicles	12.14 ± 0.36	12.30 ± 0.25	12.26 ± 0.35	11.98 ± 0.33

* – Significant differences from the results for the intact group ($p < 0.05$)

The heart mass coefficient is significantly reduced in all types of traumatic exposure relative to intact control, although there are no significant differences in this indicator among the test groups.

Traumatic exposure itself and traumatic exposure with further Levomecol treatment reduce the spleen mass coefficient; however, differences with the results for the intact group are not statistically significant at $p < 0.05$. The use of CeO_2-containing hydrogel restores the indicators to values that are not statistically distinguishable from those of the intact group.

The liver mass coefficient in different groups did not give reliable differences. The mass distribution of the remaining internal organs in the groups was consistent with the normal values for intact animals.

Analysis of the mass coefficient values did not reveal any significant differences both between the groups of animals treated with Levomecol ointment or CeO_2-containing hydrogel as well as with respect to the control group.

4. Conclusions

Thus, the experimental data obtained show that CeO_2-containing hydrogel reduces the healing time of a planar full-thickness skin wound in rats in comparison with control and reference preparation, provides rapid reduction of wound defect area and formation of a complete scar. The CeO_2-containing hydrogel has a greater effect than Levomecol ointment on non-specific signs of the inflammatory process. Thus, CeO_2-containing hydrogel normalizes body temperature, improves morphological composition of peripheral blood, leukocyte formula, and mass coefficients of internal organs of experimental animals.

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