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Cerium Dioxide Nanocrystal (Nanoceria): A Promising Pioneer Therapeutic Agent In Wound Healing Process.

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ABSTRACT

Wound healing is a complex and dynamic process of replacing disabled and missing cellular structures and tissue layers. Accelerated wound healing requires a harmonized cellular response involving different cells. In this outlook, we call attention to the most contemporary developed nanotechnology-based therapeutic agents, Nanoceria, and discern the viability and influences of it with focus on full-thickness excisional wounds. Due to Nanoceria's multiple impressive functions (Anti-inflammatory, Antioxidant, Skin protection, Antimicrobial, etc.), it had gained exclusive attention for scientists to take this unique compound into consideration. This work examines the effect of Nanoceria on wound healing process by measuring SH-group content, pro-/anti-oxidant system, antimicrobial impact and wound area reduction. As our results demonstrated, when nanoceria was applied to the wounds, reduction of TBARS products, level of schiff bases and conjugated dienes and catalase activity were observed. In addition,we observed the restoration of SOD activity and sulfhydryl groups. Moreover, "Nanoceria" has a strong bactericidal effect on *S. epidermidis, S. aureus, P. aeruginosa, C.albicans* that makes the appropriate application of the drug for the treatment of infectious inflammatory processes and in a test culture of *Candida* yeasts Nanoceria produced fungistatic effect. By analysing the active contraction of the wound surface in dynamics, we have seen in experimental group the time of full repair decreased by 13.0% ($p<0.05$) compare to the intact. It was concluded that a simple topical application of water soluble Nanoceria speeds up the healing of full-thickness wounds and makes a promising tool for further investigations.

Keywords: SH-group, GSH, TBARS, Schiff base, LPO.

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INTRODUCTION

Wound healing in skin is an evolutionarily sustained, highly interconnected as well as regulated process. It takes place over the incessant yet overlapping phases of hemostasis, inflammation, proliferation, and remodeling [1, 2]. The skin is the organ most challenged by multiple extrinsic stress factors, resulting in frequent cell and barrier damage. As such, skin has constructed a set of complex mechanisms to safeguard itself and to restitute tissue entirety when damaged, without resulting in septicemia [1]. Demographically, the number of patients enduring chronic wounds and bad healing conditions is reaching epidemic ratio and will become even more severe in both human health and economic terms [3, 4]. Penurious wound healing after trauma, surgery, acute illness, or chronic disease conditions affects millions of people worldwide each year and is the consequence of poorly regulated elements of the healthy tissue repair response, including inflammation, angiogenesis, matrix deposition, and cell recruitment [1] The failure of many recent approaches to render specific results such as wound closure, control of fluid loss, and presenting properties such as durability, elasticity, and histocompatibility has conducted to the introduction of numerous nano-technological advances [5]. Nano-therapeutic approaches that employ materials engineered with at least one dimension within the nano-scale (1−100 nm) were pioneered to efficiently control wound healing and underrate any possible complexity that might rise during this procedure [5, 6]. As a result, nanoparticles have an capability to deliver a maintained and controlled deliverance of therapeutics that eventuates in an accelerated healing process [7]. The major advantage of nano-materials over their bulk peers is the versatility and tenability of the nanomaterial's physicochemical features (e.g., hydrophobicity, charge, size) [8].

Cerium is a rare earth metal that, when incorporated with oxygen, can adopt a fluorite crystalline net structure that has a extremely reactive surface area for neutralization of radicals. In addition, nanoceria can reversibly bind oxygen and shift oxidation states (Ce3+/Ce4+) depending on the situations [9]. The use of nanoceria for therapeutic goals provides several benefits over other novel antioxidant approaches. For example, the delivery of nanoencapsulated antioxidant enzymes, such as SOD or catalase, has the restriction that only one sort of reactive oxygen species can be scavenged by each enzyme, whereas, several species are involved in neurodegenerative diseases [10] and nanoceria have been shown to decline their levels [11, 12]. Because of these properties, nanoceria have been investigated in biological systems and shown to exhibit antioxidant effects in various models of disease [13]. In contrast, nanoceria exhibits both catalase and SOD-mimetic activity with SOD-mimetic catalysis overpass that of the endogenous enzyme [14, 15]. One study showed that 5.8 µM nanoceria was equivalent to 527 U of SOD [16]. There are two categories of thoughts on the SOD-mimetic, hydrogen peroxide catalase mechanism of ceria. The first is that the Ce3+/Ce4+ ions, interact directly to both neutralize (i.e. oxidize) superoxide and overthrow peroxide. We will name this the ionic mechanism. The second school of thought is that both reactions proceed by oxygen vacancy creation and annihilation (filling), with the cerium ionic states interchanging between plus three and plus four to incorporate the oxygen vacancy population [17]. Returning now to the ionic mechanism the SOD mimetic component is believed to be facilitated by an enhancement in the Ce3+/Ce4+ ratio 110 while the catalase component is facilitated by a decline in this ratio [18].

In the present study, we have examined cerium oxide nanoparticles as a therapeutic agent on wound healing process by selecting white laboratory male rats that full-thickness wounds were performed on them. Our results demonstrated that applying nanoceria as a wound dressing material significantly accelerated the wound healing mechanism by acting as a powerful antioxidant and inhibiting microbial growth and basically controlling the oxidative stress level in cellular matrix.

MATERIALS AND METHODS

Preparation of Nanoceria solution

The wound dressings were put together by electrospinning. The film comprising 0.05% CeO2 (dissolved in 0.5% Carbopol) nanoparticles was elected as the optimal dressing for the in vivo study on full-thickness excisional wounds of rats. A peerless feature of these nanocrystals is that they can be applied multiple times: over weeks, cerium (IV) rich particles leisurely turn over to their initial cerium (III) content. In approximately all cases, the particles subsist colloidally firm (e.g. non-aggregated) and could be applied multiple times. An in vivo study represents Nanoceria evidence in mouse tissues with no pathogenicity. Taken together, it is suggested that cerium oxide nanoparticles are well sustained in mice and are agglutinated into

cellular tissues. The study illustrated that after 2 weeks, the wounds treated with the CeO2 nanoparticle-containing dressing attained a remarkable closure to nearly 100%. Our results delivered evidence supporting the feasible applicability of CeO2 nanoparticle-containing wound dressing for a favored wound treatment as it hastens complete wound closure and diminishes wound area in comparison with non-treated animals.

Animal Model

Research was administered on white laboratory male rats weighing 200 - 250 g, which were divided into four groups: control group (without any wound), intact group (wounded animals without any dressing application), experimental group (wounded animals with nanocria application) and carbopol group (wounded animals with carbopol application). Keeping animals and experiments were conducted according to ethical principles adopted by Ukraine First National Congress on Bioethics, international agreements and national legislation in this area [43]. Before the experiment, the rats were retained in quarantine and marked. Before performing the full-thickness wound model, animals were anesthetized by sodium thiopental (BiochemieGmbH / Austria), at a dosage of 50 mg / kg. The animals of experimental group were treated with "Nanoceria-Gel" which contains 0.05% CeO2 (dissolved in 0.5% Carbopol) nanoparticles for wound dressing. In the intact series wound healing happened without drug and only Carbopol while the control group was remained untreated .Before the experiment epilation was carried out on the back area after anesthetizing rats and one full-thickness wounds of 1 x 1 □□^{2} was formed in the skin of each mouse using surgical scalpel and forceps. Mice were treated with Nanoceria solution applied directly to the wound site once daily until healing. Statistical analysis of data was carried out by the & quot;Statistica 8.0" software package. Shapiro-Wilk's W criterion was used for the investigation of the data distribution type. Post-hoc analysis included Student's t-test for parametric data.

Pro-/Anti-oxidants system

To study pro-oxidant system, we have done TBARS assay, Anisidine value (Schiff base absorption) and conjugated dienes measurement to evaluated lipid peroxidation contents and oxidative stress level, following investigation of anti-oxidant properties measuring Catalase and Superoxide Dismutase (SOD) activities. The results have shown that the wound development was accompanied by disruption of pro-/anti-oxidant balance in blood serum which was controlled after application of nanoceria.

Pro-oxidants (Lipid peroxidation contents)

Lipid peroxidation has been described as the oxidative degeneration of polyunsaturated lipids by free radical reactions [26]. Various methods for measurement of lipid peroxidation have been created [26, 27]. Lipid peroxidation is usually estimated by measuring the major initial peroxidation products (conjugated dienes, lipid hydroporoxides) and/or minor breakdown products (malondialdehyd, hexanal, volatile hydrocrbons) [27].

TBARS assay

Thiobarbituric acid reactive substances - TBARS - are made as a byproduct of lipid peroxidation (i.e. as degradation products of fats) which can be indicated by the TBARS assay using thiobarbituric acid as a reagent. Because reactive oxygen species (ROS) have extremely short half-lives, they are difficult to measure directly. Instead, what can be measured are several products of the damage produced by oxidative stress, such as TBARS [28]. Assay of TBARS measures malondialdehyde (MDA) existing in the sample, as well as malondialdehyde produced from lipid hydroperoxides by the hydrolytic conditions of the reaction [29]. MDA is one of several low-molecular-weight end products formed via the decomposition of certain primary and secondary lipid peroxidation products.

Anisidine value (Schiff base absorption)

Schiff base is a compound that is being used as ligands to form harmonized complexes with metal ions. Conjugated Schiff bases absorb strongly in the UV-vis region of the electromagnetic spectrum. This absorption is the basis of the anisidine value, which is a measure of oxidative deterioration for fats and oils. p-

Anisidine condenses readily with aldehydes and ketones to form Schiff bases, which absorb at 350 nm. This colorimetric reaction is used to test for the presence of oxidation products in fats and oil [30].

Conjugated dienes measurement

It is generally accepted that the appearance of conjugated dienes in lipids stands for autoxidation of lipids. In fact, because of the divinylmethane structure, PUFA (polyunsaturated fatty acids) are particularly influenced by hydrogen removal by free radical attack, becoming themselves free radical intermediates. This results in the rearrangement of the double bond to conjugated dienes and, in the attendance of O2, the development of fatty acid hydro-peroxides [32]. The methods commonly used for specifying conjugated dienes are based on measuring their absorption at 233-236 nm [26, 33, 34]. The conjugated diene moiety is a powerful chromophore that can be indicated spectrophotometrically. When existing in fatty acids they display a characteristic absorption in the UV region [32].

Measurement of SH-group content (or Glutathione assay)

For glutathione (GSH) content assessment in blood serum, 5, 5'-Dithiobis (2-nitrobenzoic acid) (DTNB) was used as the indicator. Cells were analyzed with the spectrophotometric method [19]. A modest report of spectrophotometric method was carried out for the routine attendant determination of sulfhydryl groups in PB-SH, NP-SH, and T-SH fractions. These spectrophotometric procedures are based on the method of Ellman (20, 21), who reported that 5, 5'-dithiobis- (2,-nitrobenzoic acid) is reduced by SH groups to form 1 mole of 2-nitro-5-mercaptobenzoic acid per mole of SH. The nitromercaptobenzoic acid anion has an intense yellow color and can be used to measure SH groups. The reaction is:

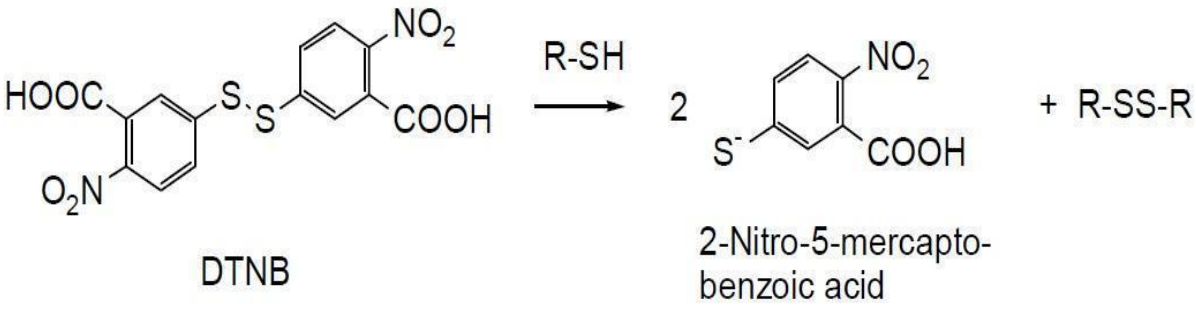

Figure 1: Reaction of Ellman's Reagent with sulfhydryls.

Ellman's reagent is used for the modification of free thiols in proteins. It quickly forms a disulfide bond with the thiol and liberates a thiolate ion which is colored (yellow). The maximal absorbance of this thiolate is at 412 nm. Its presence can be plotted against a standard curve for characterization of the total amount of free thiols in proteins. The participation of three types of GSH (Glutathione peroxidase (GSH-Px), glutathione S-transferase (GSH-Tr) and glutathione reductase (GSSG-Rx)) in the detoxification of reactive species of oxygen and in the inactivation of electrophiles such as carcinogenic-epoxide metabolites has been well established. Both types of processes can occur enzymatically. Particularly, GSH-Px assists the breakdown of H2O2 and other organic hydro-peroxides whereas GSH-Tr is responsible for the deactivation of electrophiles [22, 23]. The non-protein low molecular weight sulfhydryl groups, known as thiols, such as cysteine and glutathione play considerable functions in cells. Glutathione represented many impressive roles such as antioxidant defense, detoxification of electrophilic xenobiotics, modulation of redox regulated signal transduction, storage and transport of cysteine, regulation of cell proliferation, synthesis of deoxyribonucleotide synthesis, regulation of immune responses, and regulation of leukotriene and prostaglandin metabolism [24] (figure 2).

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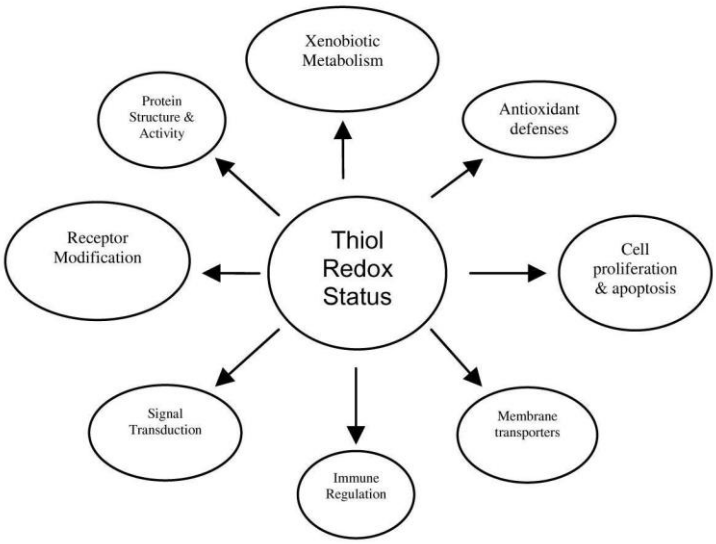

Figure 2: Thiol redox status and its many accompanying roles [24].

On the other hand, cysteine a sulfur containing amino acid has been offered as a nucleophile (i.e. the reactive center of an enzyme). Cysteine's ability to happen in up to 10 different sulfur oxidation states in vivo directs to a range of cysteine modifications in peptides and proteins. Each of these cysteine modifications represents its own particular chemical and biochemical characteristics such as stability, redox-behavior, metal-binding, acidity, nucleophilicity, and catalytic activity. r. This inimitable reactivity of cysteine is reflected by the innumerable of functions that cysteine complies in vivo, including structural stabilization, catalysis, redox-activity, and metal-binding (figure 3) [25].

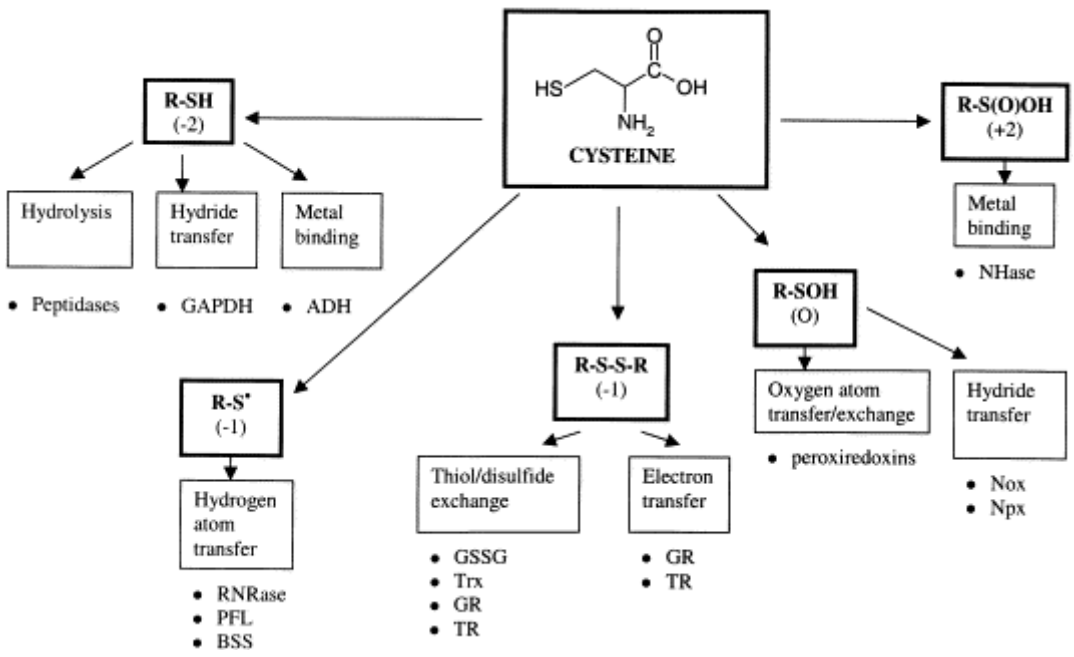

Figure 3: Oxidation states, properties, reactivity, and occurrences of different cysteine modifications in vivo. Enzymes discussed in the text are shown as examples. Oxidation states of sulfur are given for R in oxidation state +1 [25].

Antioxidants (Catalase and SOD activities)

Oxidative stress is the root of many drastic diseases and one of its primitive features is the cellular disbalance between endogenous antioxidant defenses (free radical scavenging by small molecule antioxidants

and/or redox enzymes) and ROS (e.g., superoxide radical anion, hydrogen peroxide (H_2O_2); hydroxyl radical) production inside the cells [35]. The capability of nanoceria to switch between oxidation states is comparable to that of biological antioxidants. This capability imparts nanoceria with the very important biological property of radical scavenging [36]. Researches have suggested that nanoceria possesses predominantly SOD-mimetic and CAT-mimetic activities [35].

The received data have shown that in the case of a full-thickness wound in the blood serum, products of the LPO accumulate, indicating a disturbance of the balance between the intensity of free radical procedures and the work of the antioxidant system. The obtained data indicate that the cerium-based drug contributes to the effective blockage of the LPO stages, which declares itself as a steady decrease in the content of lipid peroxidation products.

Antimicrobial efficacy

Wound healing is often accompanied by complexity of infectious nature. Infection has mostly local purulent nature, less often - putrefactive (purulent and putrid inflammation) and much less - anaerobic, spore forming (gas gangrene and phlegmon) and specific (tetanus, diphtheria). Agents of the first two forms - purulent and putrefactive – are staphylococci and streptococci, bacillus, proteus, Pseudomonas aeruginosa and anaerobic non spore forming clostridium - bacteroides etc. [37]. Therefore, the surface of wound is the most favorable healing environment for the generation of pathological microflora (which is prevailed by species of Staphylococcus spp. (42, 7%), a gram-negative microorganism from which Pseudomonas aeruginosa may be found (10, 3 %) [38]. The surface of wounds of various etiologies may be also infected with microscopic yeast fungi Candida albicans.

To study antibacterial properties of "Nanoceria" the method of application of the drug (1 g) to the surface of the nutrient medium was used (NA - Nutrientagar, manufacturer Sigma-Aldrich, Spain). Previously surface environment was covered with suspension of test microorganisms according to the recommendations (on the recommendations, "Determination of the sensitivity of microorganisms to antibiotics" Ukraine Ministry of Health, Order number 167 of 05.04.2007). Number of colony forming units (CFU) was determined by densitometer «Vitek-2» («BioMerieux» (France)). CFU load in microorganism suspensions amounted to 1,5×10 8 CFU/ml (which corresponds to 0,5 McFarland) for bacteria, 1-5×10 6 CFU/ml for yeasts. Culture collections of bacteria Staphylococcus aureus ATCC 25923, S. epidermidis 509/5, Pseudomonas aeruginosa ATCC 27853 and yeasts Candida albicans were used as test culture. We investigated the effect of nanoceria on these bacterial cultures of microorganisms.

Wound area reduction

The management of full-thickness wounds is a general part of dermatologic practice. The healing of these wounds is an intricate and interrelated series of incidents that eventually guides to both a structurally and functionally decent result. The commence of this process is demonstrated by a hemorrhage to the tissues, whether due to an unintended trauma, such as abrasions, excoriations, blisters, burns, hypothermic injuries, ischemia, or numerous other etiologies, or a defect that has been planned, such as a surgical incision or piercing. The sequence that follows leads to the repair and restoration of the site in question. Generally, there are 4 stages seen in the healing of any wound: hemostasis, inflammation, proliferation or granulation, and matrix formation or remodeling [39-42].

Statistical analysis

Statistical analysis of data was carried out by the "Statistica 8.0" software package.The type of in-group data distribution was verified via the Shapiro-Wilk test. As data were distributed normally ($p > 0,05$), two-way ANOVA was conducted to determine the significance of difference between means, with Bonferroni post test. Difference between means was judged as statistically significant if $p \leq 0,05$. Mean and standard deviation (SD) were calculated for each group.

RESULT AND DISCUSSION

Overall, this experiment demonstrated the development of full-thickness wound that is accompanied by an increase in the processes of lipid peroxidation in the blood serum of rats. This is manifested by enhancement in the content of LPO products: conjugated dienes, TBARS compounds and schiff bases, which are indicators of the development of oxidative stress. This changes the activity of antiradical enzymes: superoxide dismutase activity decreases and catalase increases, which is associated with the intensification of the formation of active forms of oxygen. Also, under these conditions in serum, a decrease in the level of protein and non-protein sulfhydryl groups is observed, indicating that they are damaged by free radicals. It was found that in the case of full-thickness wound under conditions of nanoceria application in experimental group of rats, the oxidative-antioxidant balance is restored in serum, as shown by the decrease in the products of the LPO and the normalization of antiradical enzymes.

The discussion is given below. According to the measurements:

TBARS assay: in intact group of animals, the content of TBARS compounds increases: on 3rd day by - 2.1 ($p < 0,05$), 6th day - 3 ($p < 0,05$) and on 20th day by - 1.3 ($p < 0,05$) compared with the control group. The content of TBARS compounds in the blood serum of wounded rats under the action of carbopol was similar to that of intact group. In the blood serum of experimental group of rats, it was shown that when applying nanocria, the level of TBARS products decreased on 3rd and 6th and 20th days. Their content was below the reference level by 1.5 ($p < 0,05$) (table 1).

Table 1: Content of TBA-active products in blood serum of rats with full-thickness, nmol × mg of protein-1(* - p <0,05 - compared to the control group of animals).

Time(days)		Indicator
Control		14,73 ± 1,32
3rd	Intact	31,41 ± 3,09*
	Carbopol	29,07 ± 2,83*
	Nanoceria	23,12 ± 2,17*
6th	Intact	44,18 ± 4,29*
	Carbopol	40,18 ± 3,83*
	Nanoceria	25,42 ± 2,51*
20th	Intact	18,61 ± 1,78*
	Carbopol	19,54 ± 1,92*
	Nanoceria	9,81 ± 0,96*

Anisidine value (Schiff base absorption): We have shown in the intact group of animal, the content of schiff bases increased on for 3rd day by - 1.5 ($p < 0,05$), 6th day by - 1.8 ($p < 0,05$) and on 20th day by1.3 ($p < 0,05$) compared to the control group. Under the influence of carbopol, the level of schiff bases in the blood serum of wounded rats had similar changes of intact group. When using nanoceria, it was observed that in the blood serum of wounded rats, the level of schiff bases on 3rd, 6th and 20th days is reduced. Their content was 1.7 ($p < 0,05$) lower than the control level (table 2).

Table 2: The content of schiff bases in blood serum of rats with full-thickness wounds, µmol × mg of protein^{-1} (* - p <0,05 - compared to the control group of animals)

Time(days)		Indicator
Control		15,22 ± 1,43
3rd	Intact	23,41 ± 2,32*
	Carbopol	25,36 ± 2,52*
	Nanoceria	18,82 ± 1,87
6th	Intact	27,87 ± 2,74*

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	Carbopol	29,12 ± 2,87*
	Nanoceria	20,38 ± 2,01*
20th	Intact	19,61 ± 1,88*
	Carbopol	18,76 ± 1,85*
	Nanoceria	8,92 ± 0,87*

Conjugated dienes measurement: The results have shown that in the blood serum of intact group of animals, the content of LP products increases. Thus, the content of conjugated dienes increases: on 3rd day - 1.5 ($p < 0,05$), for 6th day - 1.6 ($p < 0,05$) and on 20th day the level of conjugated dienes decreases to the control level. The content of conjugated dienes in the blood serum of wounded rats under the action of carbopol had changes similar to the intact group (table 3). When using nanocria on experimental group of rats, there is a decrease in the level of conjugated dienes, and on 20th day their content was lower than the control level of 1.7 ($p < 0,05$).

Table 3: The content of conjugated dienes in blood serum of rats with full-thickness wound, µmol × mg of protein^{-1} (* - $p < 0,05$ - compared to the control group of animals).

Time(days)		Indicator
Control		1,23 ± 0,12
3rd	Intact	1,81 ± 0,17*
	Carbopol	1,95 ± 0,19*
	Nanoceria	1,75 ± 0,17*
6th	Intact	1,97 ± 0,19*
	Carbopol	2,12 ± 0,19*
	Nanoceria	1,51 ± 0,15*
20th	Intact	1,51 ± 0,15
	Carbopol	1,62 ± 0,16
	Nanoceria	0,73 ± 0,07*

Antioxidants (Catalase and SOD activities): We represented in the intact group of animal the activity of enzymes in the first line of cell protection from oxidative stress changes: superoxide dismutase activity decreases and catalase activity increases with respect to the control group of animals. Thus, superoxide dismutase activity is reduced on the 3rd day - by 9.1 ($p < 0,05$), 6th day - by 6.6 ($p < 0,05$) and on 20th days - by 6.1 ($p < 0,05$) compared to the control group. In this case, the catalase activity increases on 3rd day - by 2.9 ($p < 0,05$), on 6th day - by 3.3 ($p < 0,05$) and on 20th day - by 1.2 ($p < 0,05$) compared with the control group. The activity of antiradical enzymes in blood serum of rats with an experimental wound under the influence of carbopol was similar to that of intact
group. With the use of nanoceria on experimental group, restoration of superoxide dismutase activity and reduction of catalase activity to the control level are observed (table 4).

Table 4: Activity of antiradical enzymes in blood serum of rats with full-thickness wounds (* - $p < 0,05$ - compared to the control group of animals).

Time(days)		SOD activity conv. unit × min^{-1} × mg of protein^{-1}	Catalase activity conv. unit × min^{-1} × mg of protein^{-1}
Control		2,19 ± 0,22	4,21 ± 0,41
3rd	Intact	0,24 ± 0,02*	12,13 ± 1,19*
	Carbopol	0,27 ± 0,02*	14,26 ± 1,38*
	Nanoceria	1,59 ± 0,15*	7,22 ± 0,67*
6th	Intact	0,33 ± 0,03*	14,08 ± 1,38*
	Carbopol	0,38 ± 0,03*	18,21 ± 1,79*
	Nanoceria	1,52 ± 0,15*	8,73 ± 0,85*
20th	Intact	0,36 ± 0,03*	5,09 ± 0,51

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	Carbopol	0,39 ± 0,03*	5,64 ± 0,55
	Nanoceria	1,65 ± 0,06*	4,83 ± 0,46

Measurement of SH-group content (or Glutathione assay**):** Also, under these conditions in serum, a decrease in the level of protein and non-protein sulfhydryl groups is observed, indicating that they are damaged by free radicals. According to our results in the blood serum of intact group of rats, the content of sulfhydryl groups is reduced. Thus, the level of non-protein SH-groups is reduced on 3rd day - 1.8 (p <0,05), 6th day 1.9 (p <0,05) and by 20th day by 1.4 (p <0,05) compared to the control group. In this case, the content of protein SH groups is reduced on 3rd day by 1.3 (p <0,05), 6th day - 1.6 (p <0,05) and 20th day - 1.4 (p <0,05) compared to the control group. Similar changes in the level of sulfhydryl groups have been found in the blood serum when investigating the effects of carbopol.

In the experimental group of animals, the level of total-protein SH-groups is reduced on 3rd day by-1.3 (p <0,05), 6th day - 1.2 (p <0,05) and on 20th day - 1.4 (p <0,05) compared to the control group. It was demonstrated when nanoceria was applied to wounds, the restoration of sulfhydryl groups was observed (table 5). The obtained results indicate that the level of free radicals increases in serum on the wounded surface area, which leads to the depletion of the level of non-protein low molecular weight thiols (cysteine, glutathione, etc.) and inhibition of the activity of thiol enzymes by blocking their sulfhydryl groups (glutathione peroxidase, glutathione transferase, glutathione reductase). Reduction of the total, protein and non-protein SH groups in this experiment (intact group) reflects the overall displacement of the redox-balance in the pro-oxidant side.

Table 5: The content of sulfhydryl (SH-) groups in blood serum of rats with full-thickness, µmol × mg of protein^{-1} (* - p <0,05 - compared to the control group of animals).

Time(days)		NP-SH group	P-SH group	T-SH group
Control		0,27 ± 0,02	4,52 ± 0,43	4,79 ± 0,46
3rd	Intact	0,15 ± 0,01*	3,41 ± 0,33*	3,56 ± 0,34*
	Carbopol	0,14 ± 0,01*	3,22 ± 0,31*	3,36 ± 0,33*
	Nanoceria	0,17 ± 0,01*	3,48 ± 0,34*	3,65 ± 0,36*
6th	Intact	0,14 ± 0,01*	2,81 ± 0,27*	3,95 ± 0,38*
	Carbopol	0,13 ± 0,01*	2,55 ± 0,25*	3,68 ± 0,36*
	Nanoceria	0,21 ± 0,02*	4,36 ± 0,41	4,57 ± 0,35
20th	Intact	0,19 ± 0,01*	3,17 ± 0,31*	3,36 ± 0,32*
	Carbopol	0,18 ± 0,01*	3,03 ± 0,29*	3,21 ± 0,31*
	Nanoceria	0,29 ± 0,03	4,85 ± 0,46	5,14 ± 0,51

Antimicrobial efficacy:

studying biocidal effect of Nanoceria, it was revealed on test cultures of bacteria Staphylococcus aureus and Pseudomonas aeruginosa. In a test culture of Candida yeasts Nanoceria produced fungistatic effect (areas of growth retardation were noted, where a decreased intensity of yeast growth was observed) (figure 4).

"Nanoceria" has a strong bactericidal effect on S. epidermidis, S. aureus, P. aeruginosa, C.albicans that makes the appropriate application of the drug for the treatment of infectious inflammatory processes. The degree of Nanoceria influence in test cultures of microorganisms was evaluated by the presence or absence of growth inhibition zones and their size (diameter). Variants of experiments using gel containing carbopol only (without nanoceria) and options without making any drugs served as control options.

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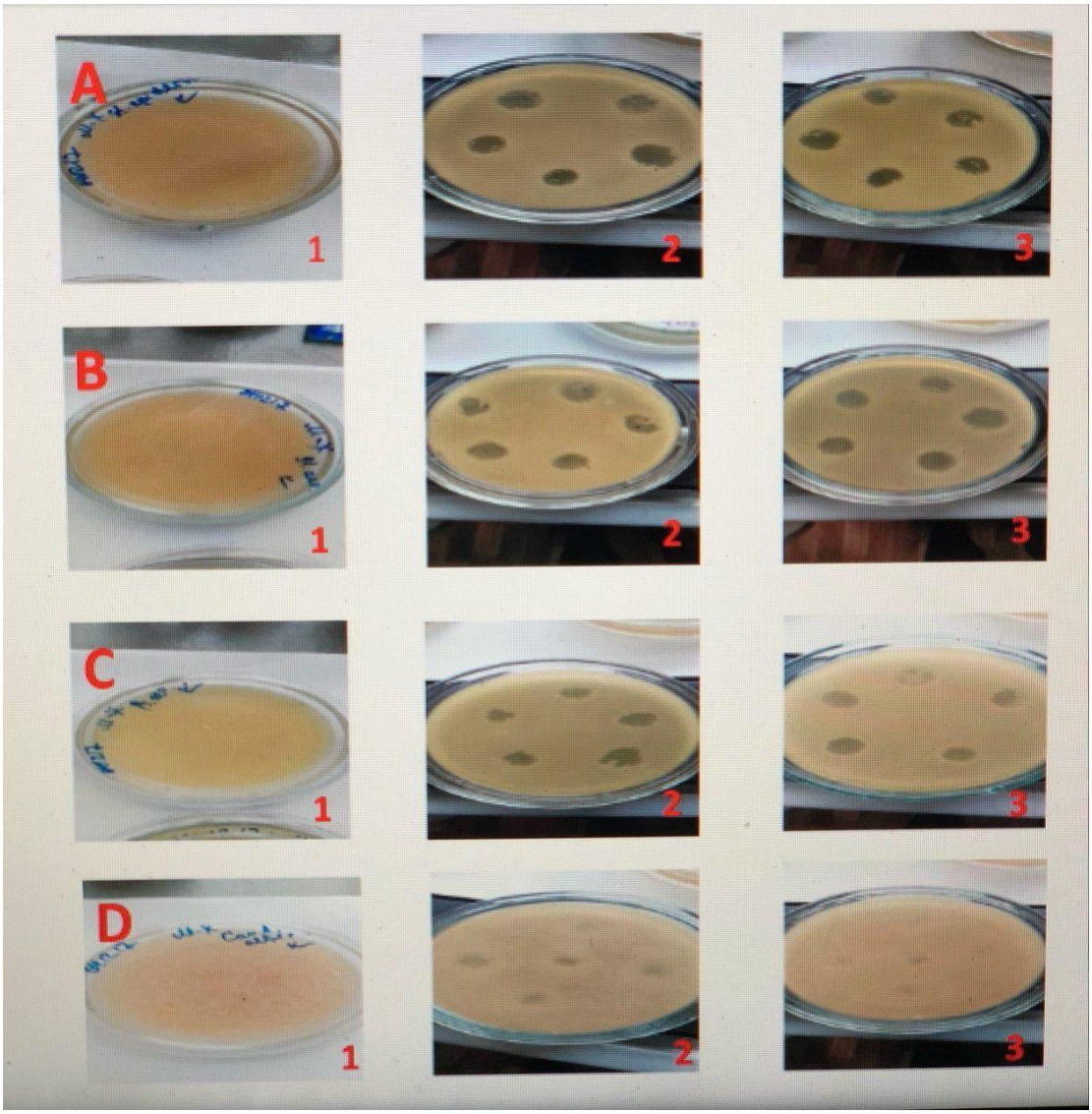

Figure 4: Bactericidal action of nanoceria on the bacteria Staphylococcus epidermidis (A), Staphylococcus aureus (B), Pseudomonas aeruginosa (C), Candida albicans yeasts (D). Ingredients: 0.05% nanoceria, 0.5 % carbopol. A, B, C: 1- control, 2.3 - growth inhibition zones in the places of application of Nanoceria (back side of the Petri dish). The density of the slurry 1,5× 10^8 CFU / ml.24 hours of cultivation. ,D: 1 - Control, 2 - 24 hours of cultivation, 3 - 72 hours of cultivation, inhibition area of growth over grew (back side of Petri dishes).The density of the slurry 1-5× 10^6 CFU/ml.

Wound area reduction: evaluation of healing effect of the studied drug "Nanoceria" was conducted by analysis of active contraction of the wound surface in dynamics On 3rd, 6th, 9th, 14th day and after complete wound closure 8 rats from each group were sacrificed, photos were made and exact area of the wound was measured (figure 5).

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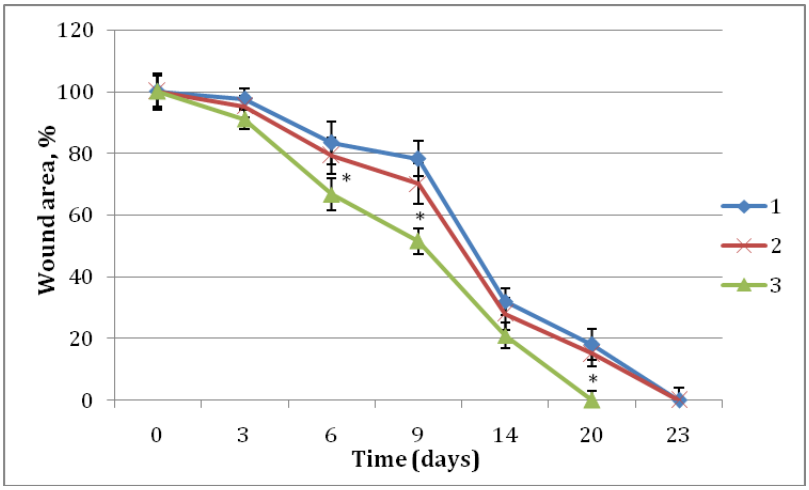

Figure 5: The surface of full-thickness wound area with Nanoceria action (% of the original size) , M±m (n=8 in each group of animals) / * - p<0.05 compared with control group

1 - Intact **(Group without drug);**
2 - Carbopol (Group without drug and only Carbopol);
3 - Experimental (Group treated with Nanoceria)

In the model of full-thickness skin wound it was shown that complete wound closure in the intact group of animals occurred on the 23.0±0.8 day (figure 5, 6). In experimental group the time of full repair decreased by 13.0% ($p<0.05$) compare to the intact and was equal to 20.0±0.5 day. On 6th, 9th and 20th day of experiment Cerium dioxide treated wounds areas were by 20.1% ($p<0.05$), 19.8% ($p<0.05$) and by 34.0% ($p<0.05$) accordance decreased in comparison with control. In other time periods the rate of healing was approximately the same. On the 9th and 14th day of the experiment in the above mentioned group healing was more intense compared with the intact group.

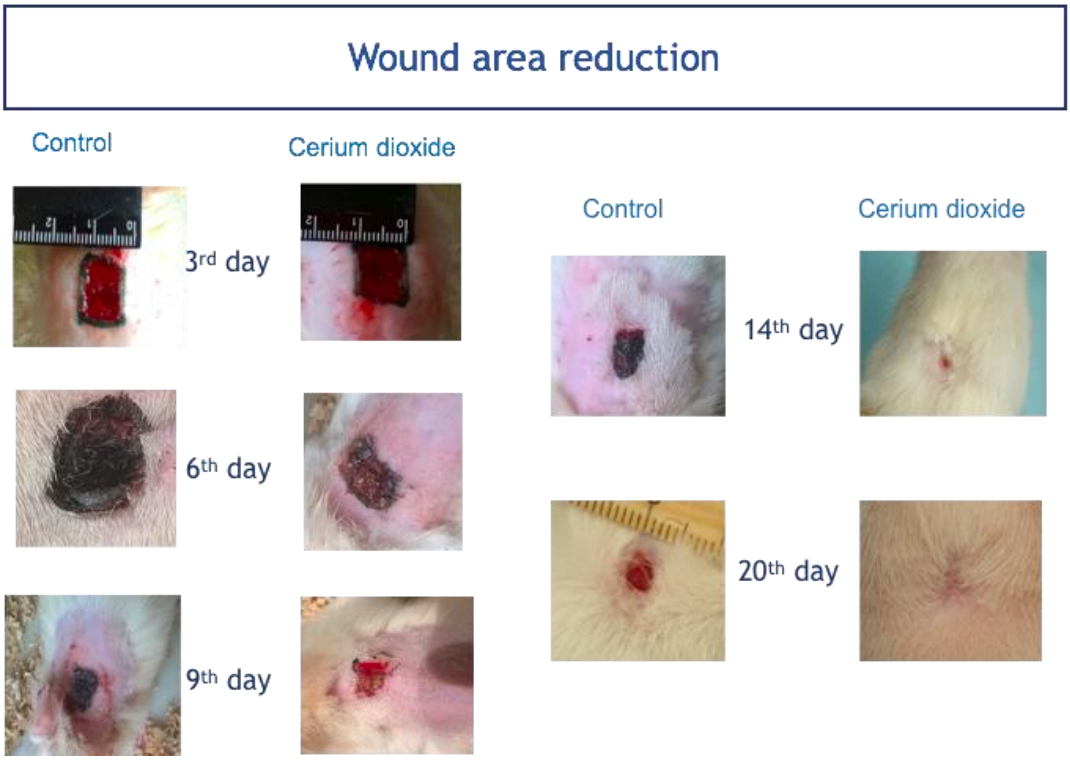

Figure 6: The appearance of the full-thickness surface intact and experimental groups in different time (days) of experiment.

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CONCLUSION

In summary, we have shown that a simple topical application of water soluble Nanoceria speeds up the healing of full-thickness dermal wounds in a rat model. Moreover, Nanoceria decreases the oxidative stress in wounded region and defends regenerative tissue. Basically, nanoceria due to its dual oxidation state could promote wound healing activity when compared to standard drug by salvaging ROS from the location of injury and protecting the native tissue by helping in producing high amount of collagen which is a marker for effective wound healing. Furthermore, "Nanoceria" has a strong bactericidal effect S. epidermidis, S. aureus, P. aeruginosa, C.albicans, that makes the appropriate application of the drug for the treatment of infectious inflammatory processes.

This study indicates the therapeutic potential for topical treatment of wounds with regenerative engineered antioxidant Nanoceria particles. According to results we consider nanocrystal Cerium dioxide a perspective and promising drug for further investigations.

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