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NANOMATERIALS BASED ON CERIUM OXIDE NANOPARTICLES FOR WOUND REGENERATION: A LITERATURE REVIEW

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Objective: to analyze data on the synthesis and properties of cerium oxide nanoparticles, as well as the prospects of its application in regenerative medicine for wound healing. **Methodology.** World literature was reviewed using PubMed, SCOPUS, ResearchGate, CyberLeninck, and Elibrary databases, as well as manual searches for authors and reference lists. Key search terms were "cerium oxide" AND nano* AND (healing OR regeneration OR repair) AND wound". The timeline was from the date of publication through August 2023. **Results.** The final analysis included 59 sources containing information on the synthesis and size of nanoparticles (and/or other physicochemical characteristics), methodology and results of in vivo and in vitro studies on the efficacy and/or safety of nanoceria for wound regeneration. It is shown that despite the progressive growth of research interest over the last 15 years, the actual use of nanoceria in practical medicine is still not widespread. This is due to a wide variety of non-standardized synthesis methods and conditions, resulting in the variability of physicochemical parameters of nanoparticles (size, form), thereby affecting the safety and biomedical efficacy of nanoceria. Regeneration mechanisms, including the antioxidant-prooxidant, anti-inflammatory and antimicrobial effects of nanoceria, which contribute to accelerated wound healing, are discussed. The severity of the regenerative effects depends on the method and conditions of synthesis, hence the resulting physicochemical characteristics of the nanoparticles. Therefore, after each batch, newly synthesized nanoceria needs physicochemical and biomedical experimental tests. **Conclusion.** Nanoceria has great potential in tissue engineering for regenerative medicine, particularly for healing of various kinds of wounds. Having developed a technology for standardized synthesis for effective and safe nanoceria (of the right form and size) on a production scale, it can be introduced in medicine, possibly improving the outcomes of treatment of many diseases and pathologies. The authors present conclusions on the results of the study of nanoceria for accelerating qualitative regeneration and the requirements for nanoparticles obtained during synthesis.

Keywords: nanomaterials, nanoparticles, cerium oxide, regeneration, regeneration mechanisms, nanoceria, nanoceria synthesis, size and form, coating, antioxidant effect, antibacterial activity, safety, skin wound healing.

INTRODUCTION

Throughout the history of medicine, issues about wound healing have always attracted robust discussions. This is not surprising because skin wounds still occupy a leading position among all pathologies that require the attention of a doctor. Over such a long period of time, many methods for accelerating the regeneration of damaged skin have been proposed. Natural plant and animal substances, synthetic substances with antimicrobial and regenerative properties, auto- and allogeneic progenitor cells have been used. In the last decade, due to the advent of technologies for the production of nanosized metal salt particles and in connection with some biostimulatory effects inherent in nanoparticles, researchers have shifted their focus to this area. One of the compounds that have demonstrated its ability to stimulate tissue regenerative mechanisms, antioxidant and bacteriostatic activity is cerium dioxide.

Cerium oxide nanoparticles (CeO_2-NPs, nanoceria) have long been studied in biomedical research. According to the international database PubMed, studies on nanoceria have been published for the last 27 years (2281 in number). While the number of such works was in units in the period up to 2005, it was in tens in the period 2005–2009 and in hundreds since 2010. In 2022, 407 publications on nanoceria were registered in the PubMed database. The attractiveness of nanoceria application for regenerative medicine is down to its biosafety, as well as its antimicrobial, angiogenic, and proliferative properties with respect to all cell lines involved in skin regeneration, and antioxidant properties [1–7].

One of the most important characteristics of nanoceria is its redox activity due to its ability to exhibit a trivalent or quadrivalent state depending on the pH of the environment. This makes it unique and extremely reactive. Many researchers have noted this exceptional ability

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of nanoceria to switch between oxidation states Ce^{4+} and Ce^{3+}, coexisting on the surface of cerium nanoparticles, depending on the properties and state of the environment, which accounts for its redox-active pro- and antioxidant properties [5, 8–12]. An important feature is the ability of cerium to return the original valence value by adding or removing oxygen atoms with minimal structural reorganizations, which allows the nanoparticle to be used repeatedly in redox reactions [13].

However, despite the progressive increase in research interest, the actual application of nanoceria in practical medicine is still not widespread. There are reasons for this. First, there are several synthesis techniques available now, and production conditions directly affect the final physicochemical parameters of nanoparticles, and hence the (bio)medical result [4]. Secondly, scientific debate on the active properties of nanoceria continues. Regenerative, antibacterial, antioxidant potentials – each of these characteristics has studies confirming or refuting them. Finally, there are various conflicting data on the toxicity of this compound; some works have recorded no death of normal cells up to high concentrations of the substance, while some other reports have shown signs of apoptosis and genomic disorders even at minimal doses. It is likely that the second and third problems are related to the first, since the ambiguous (and sometimes opposite) conclusions by different authors are associated with initially different physical and chemical properties of synthesized nanoceria. This means that only if patterns of influence of the physicochemical characteristics of nanoceria at the molecular, cellular, tissue, organ and, finally, organismal level can be determined that, having developed a technology for standardized synthesis of the most effective and safe nanoceria, with a high degree of probability, its use can be introduced in biology and medicine, possibly improving treatment outcomes for many diseases and conditions.

The aim of this work is to systematize available literature and analyze the data on synthesis and properties of CeO_2-NPs, as well as the prospects for their application in regenerative medicine.

METHODOLOGY

We conducted a review of the world literature using PubMed, SCOPUS, ResearchGate, CyberLeninck, Elibrary, and manual searches by authors and reference lists. The search query used was “cerium oxide” AND nano* AND (healing OR regeneration OR repair) AND wound”. There were no restrictions on publication time (from first publication until August 2023). Inclusion criteria were study material (cerium oxide nanoparticles), skin wound model or other components of regeneration (cell proliferation, migration, scratch test). Types of work: original study, review, meta-analysis, bibliographic analysis, systematic review. Exclusion criteria: micro- or macroceria study.

RESULTS

After analyzing the lists found and removing repetitions, 59 publications were selected for further analysis; 140 sources did not fit the topic of biology/medicine or did not contain information on synthesis and/or size of nanoparticles, or there were no full-text articles (including authors did not send upon request). The literature selection methodology is presented in Fig. 1.

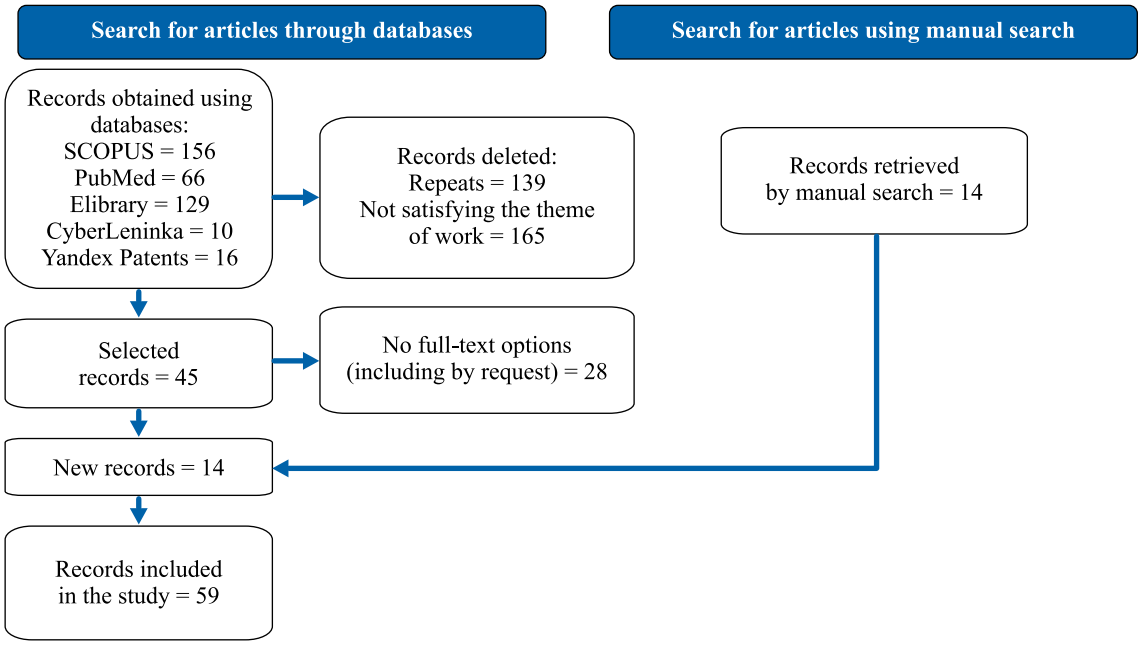

Fig. 1. Summary on the number of articles found by search engines in various databases using the search query “cerium oxide” AND nano* AND (healing OR regeneration OR repair) AND wound” from the date of first publication through August 2023

Out of nearly 200 papers, only a quarter of them contained information on physicochemical (at least 1) properties, method of synthesis (at least briefly), study methodology, and in vitro or in vivo results. However, due to a number of limitations, which are presented here, there is no clinical use of nonoceria yet.

Selection of synthesis technique

To begin with, it is worth dividing CeO_2-NPs synthesis techniques into the following main ones (according to the nature of the synthesis process): physical, chemical and green synthesis. These processes are completely different. Therefore, the results obtained when using the different techniques will be different. In recent years, hybrid (e.g., mechanochemical) synthesis methods combining the first two options have also been emphasized, which is even more associated with the problem of obtaining different results.

The physical method of nanoceria synthesis, which is based on physical processes such as mechanical grinding, melt atomization, and physical vapor deposition, is characterized by a wide range of nanoparticle diameters. Although there are reports on successful synthesis of small particles in a small range (3–5 nm) and possible control of the nanoceria production process [14], however, not all works were able to obtain such results for calibrated particle size [15].

The chemical methods of nanoceria synthesis are based on chemical reactions. These include such types of nanopowder synthesis as chemical vapor deposition, deposition from solutions, high-energy synthesis, reductive processes, and others. Chemical synthesis of nanoceria is characterized by a wide variety of techniques, but among them there are the so-called industrial techniques: low-cost in terms of components and number of reactions [16, 17].

The green synthesis method, based on which metal nanoparticles are prepared immediately with organic coating using natural biological substrates (plants, bacteria, fungi, yeast), will allow obtaining additional properties of the final nanoparticle. For example, there is a synthesis method using Curvularia lunata extract that added antibacterial activity to a nanoparticle that previously did not exhibit such activity; such properties appeared with the use of pectin as a stabilizing and reducing agent [18, 19]. Also, the formed particle obtained bacteriostatic activity (at 2 mM concentration, *E. coli* survival rate 5%, *B. subtilis* 3%). Tannic acid-based synthesis increased the number of oxygen vacancies [20]. In another work, curcumin was used in a composite with nanoceria, additionally with anti-inflammatory effects; the biological efficacy when synthesized in combination with curcumin was higher than directly with cerium alone [13]. In addition, curcumin is a non-invasive indicator of the pH environment, which can theoretically control the progress of wound healing [21].

In addition to the variety of chemical and physical methods of nanoceria synthesis, there are other classifications of nanomaterials production methods: by the aggregate state of starting materials (gas-phase, solid-phase (from solid materials), liquid-phase (from solutions)); by type of nanoparticle formation and assembly technique (condensation from atoms or molecules) or dispersion (deformation), and others. All variables, including degree of temperature and duration of temperature exposure during the synthesis process, can affect the final result.

When choosing a synthesis, it is worth keeping in mind that there is evidence that processing CeO_2-NPs at high temperatures leads not only to loss of antioxidant activity but also to acquisition of pro-oxidant properties [5, 9, 22, 23].

Also, properties of the resulting nanoparticles of the same shape and size differ depending on the precursors. For example, precipitation from Ce(IV) solutions leads to formation of more stoichiometric nanoparticles compared to samples synthesized from Ce(III) salts [24].

Nanoparticle shape and size

The size and shape of the particles determine the redox activity of nanoceria, and the biological activity exhibited [22, 23], so these nanoceria characteristics are fundamental.

Currently, the following are the main synthesized shapes of nanoceria: spherical, cubic, rod-shaped, octahedral, rhombohedral, and spiked. The latter has been reported to be more effective in forming nanobridges that promote faster wound healing compared to the control [15]. Most likely, it was this with the spiky form that promoted regeneration, as the other effects (antioxidant, antimicrobial) were not significant. The biggest influence is the ratio of particle surface area to particle size. For example, smaller spherical and octahedral particles, approaching spherical, show better permeability through the cell membrane compared to larger particles due to penetration into cells through the energy-independent cellular uptake pathway [25]. Nanoparticles in the form of rods provide the best orientation-dependent interaction with cell surfaces; the rods contain increased levels of cerium in the Ce^{3+} valence. However, it was the cubic shape that had the greatest antioxidant effect, which the authors of the study attribute to the open face of the shape and the Ce^{3+}/Ce^{4+} ratio. Moreover, they suggest that Ce^{3+} rods form stable $CePO_4$ compounds and crystal $CePO_4/CeO_2$ complexes, reducing antioxidant abilities with the possibility of toxic effects [25].

Nanoparticle size matters more than anywhere else in nanochemistry. Smaller particles have the highest percentage of potentially active surface area, so agglomeration (clumping of nanoparticles) is undesirable. Moreover, according to researchers, when particle size is less than 10 nm, their toxicity decreases dramatically

due to the sharp increase in oxygen non-stoichiometry of particles and their reductive activity [24].

Nanoparticle coating

Along with unique suitable properties for use in regenerative medicine, nanoceria has several disadvantages that limit its use. The first is aggregation (agglomeration), which leads to loss of activity, and hence useful biological properties, against the background of increased toxicity. Higher $Ce^{3+/4+}$ reactivity is associated with higher toxicity. In addition, the highly reactive nature of nanoceria is associated with non-specificity of interaction, as well as with loss of stability of nanocomposites. Formation of the so-called protein corona (protein adsorption on nanoceria surface) can negatively affect interaction with cells and the nanoceria excretion processes [11, 26].

To overcome these limitations, to improve nanoceria stability, various methods of nanoparticle surface coating are being attempted. In addition, coating a nanoparticle achieves several advantages: stabilizing the shape and size, improving solubility, increasing the half-life of excretion, improving permeability and further stages of pharmacodynamics and pharmacokinetics [27]. Sometimes it increases biocompatibility and acquires additional useful qualities.

Various types of ligands can be used as shells to cover the surface of cerium nanoparticles: different types of polymers (polysaccharides, particularly dextran, polyethylene glycol, polyacrylic acid), carboxylic acids (citrate), polyoxometalates, silanes, peptides, and many others.

The choice of shell depends on initial particle data (particle stability, solubility) and the resulting effects required (potentiation of antioxidant effects, anti-inflammatory or antibacterial additional properties).

Dewberry et al. proposed placing microRNA-146a around cerium oxide nanoparticles. MicroRNA acts on the anti-inflammatory pathway NFkB, thus capturing the three major mechanisms of wound healing (anti-inflammatory, pro-angiogenic, antioxidant,) allowing for qualitative and more efficient acceleration of regeneration. However, the authors note that these nanoparticles can turn into agglomerates that decrease their biological efficacy [28, 29].

CeO_2-NPs containing polyethylenimine and glutaraldehyde interact with superoxide dismutase and catalase, increase their antioxidant potential and protect DNA and proteins from oxidative stress [18].

Dosage form

Nanoparticle delivery form plays a major role in the effectiveness of wound healing. Firstly, the form determines the penetration of nanoparticles into the area of action. Secondly, the rate and duration of release, and thus the efficacy and even toxicity of the substance all depend on the form. Table reviews the main form described in the works so far, their advantages and disadvantages.

Table

Main forms of nanomaterials synthesized to accelerate wound regeneration (healing)

Form	Advantages	Disadvantages / challenges	Source
Gel	• Easy to use on your own • Can mechanically protect the wound surface. • Does not require special skills and frequent changes • With proper choice of materials, it ensures gas exchange • Gel can act as a matrix for proliferation and migration of cells involved in skin regeneration and/or mesenchymal progenitor cells	• It is essential that the material has good biocompatibility and allows gas exchange (e.g., limitation for the use of gelatin). • Uniform release of cerium oxide nanoparticles • The consistency of the medical product should not give a feeling of discomfort, tightness, stickiness	Articles [13, 30–35] Patents [36, 37]
Sols/ solution	A relatively simple dosage form to manufacture	• CNPs are poorly soluble and form conglomerates; it is necessary to stabilize them and increase their permeability with a shell. • Liquid, needs to be applied to the wound frequently • No additional mechanical protection	[38, 39]
Skin films; Plaster/ bandages	• Convenient form • Mechanical protection • Does not require frequent changing, so does not re-injure the regeneration site	• Painless removal of plaster from the wound without damaging the wound • Gradual release of the active ingredient • Gas exchange • Slow degradation of the material, its compatibility	[19, 25, 26]
Injection	Immediately injected (under the skin)	Need for additional equipment, skills	[40]
Lyophilized sponge	Absorbs exudate	If repeated injections are required, there is a risk of introducing infection or damaging healed tissue	[41]

As we can see, the ideal form that would satisfy all needs is yet to be proposed.

Most researchers prefer the gel form, which is due to the ease of use, wider choice of a gel base (alginate, collagen, gelatin, polyvinyl alcohol, etc.) and relatively uncomplicated technology of synthesis and combination with cerium dioxide nanoparticles. Other forms are used less frequently, as this is associated with several difficulties: the technique of introducing the active substance into the carrier, synthesis of the matrix itself with uniform release of nanoparticles, ease of use for the patient, and much more.

We emphasize that all forms of both pharmaceuticals and medical products, including implants, scaffolds, and biosensors, have potential for use in regenerative medicine.

The challenges of developing scaffolds using nanomaterials to accelerate wound regeneration have not yet been sufficiently resolved. Although considering the ever-increasing number of lesions in modern warfare, the problem of treating extensive wounds with large soft tissue defects and depressions on the background of gross cosmetic defects will become among the main problems [6, 42, 43].

Effect of pH on the valence state of cerium

Fig. 2 shows the influence of the environmental condition on the valence state of cerium and hence on its abilities. Considering that the pH of a wound changes during regeneration or progressive microbial contamination, it has been suggested that nanoceria may potentiate the regenerative effect of other drugs against the background of bacteriostatic effects. Such nanoceria potential gave rise to the introduction of the term "smart" drug into medical literature [44].

Studies sometimes use the method of observing the change in color and homogeneity of the solution to determine the readiness of the compound during synthesis [34, 46]. There has been a proposal to use pH in evaluating the progress of wound healing, and curcumin added to the dosage form as a non-invasive indicator [21].

Regeneration mechanism of cerium oxide nanoparticles

Most researchers attribute the mechanism of regeneration specifically to the antioxidant effect [30, 39, 47]. Due to reduction of reactive oxygen species (ROS), which are formed during destruction of phagocytes with the formation of "oxidative burst" or "respiratory burst" [48]. Antioxidant reserves are used to reduce ROS, an inflammatory response develops, and the wound becomes chronic [49]. Effective wound healing occurs at low controlled levels of ROS.

There is also evidence that cell migration and proliferation are accelerated in a medium with CeO_2-NPs, but these effects are achieved in neutral or alkaline media due to the antioxidant properties of cerium [15, 39].

In addition, another mechanism of regeneration and stimulation of cell migration – modulation of expression of redox-sensitive genes – has been described [50]. TGF-β was increased in a medium with CeO_2-NPs, which promotes keratinocyte migration [51]. Also, low levels of ROS promote angiogenesis and re-epithelialization through vascular endothelial growth factor receptor (VEGFR) and epidermal growth factor (EGF) [30]. The increased level of VEGFR2 indicates stimulation of angiogenesis, one of the important elements of regeneration [51].

The regenerative effect of nanoceria is also associated with its antimicrobial activity. Many studies are

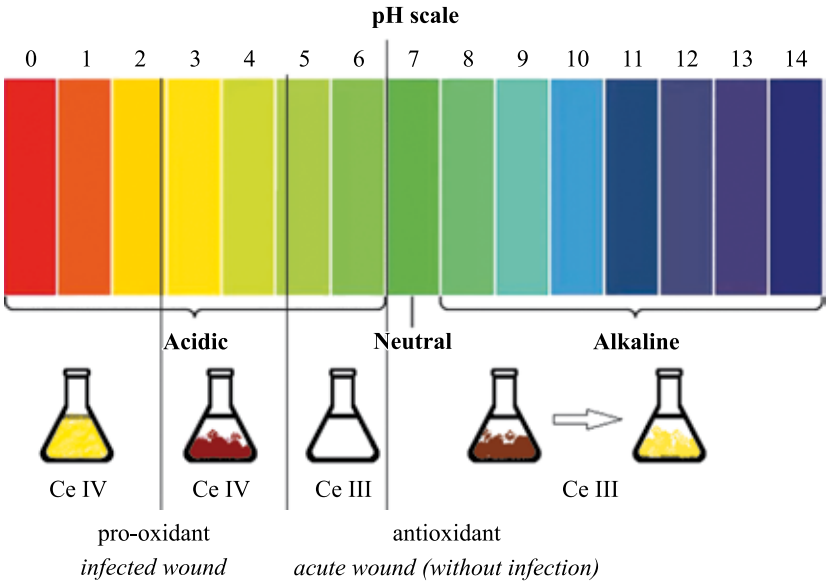

Fig. 2. Relationship between the properties of cerium oxide nanoparticles and pH of the medium [45]

devoted to the antibacterial effect of CeO_2-NPs against gram-positive and gram-negative bacteria [2, 52]. This possibility is down to the following mechanisms. The first one consists in damaging the lipid bilayer of the cell by direct contact between the nanoparticle and the membrane. As a result, the integrity of the bacterial cell is breached, its contents are lysed, and the microorganism dies. By another mechanism, the products of interaction between nanoparticles and intercellular space – ions and reactive oxygen species – have a damaging effect [24]. However, it should be noted that the second way of antibacterial activity is effective only in an acidic environment due to the peculiarities of the valence state of cerium oxide: it is in an acidic environment due to the metabolic products of microorganisms that cerium acquires a 4-valency and pro-oxidant properties with the ability to increase ROS [15].

Antibacterial activity of nanoceria

The question of severity and even presence of antimicrobial activity is still debatable. There are works claiming that nanoparticles of heavy metals or metals with variable valence have a pronounced antimicrobial effect [53–57]. At the same time, there are many works in which the authors did not obtain such an effect [30, 58]. In studies on the antibacterial effect of CeO_2-NPs, the most frequent model for experiments were strains of *Staphylococcus aureus* and *Escherichia coli* [2, 59].

However, the antibacterial effect of nanoparticles has not always been achieved. Several experiments have detected no antibacterial activity [30, 60, 61]. Meanwhile, despite not finding any direct antibacterial effect of CeO_2-NPs, P. Bellio (2018) were able to identify their synergistic effect in combination with antibiotics such as imipenem and cefotaxime [61].

Probably, the presence and severity of antimicrobial activity of nanoceria depends on the method and conditions of synthesis, hence, on the resulting physicochemical characteristics of the nanoparticles. Thus, nanoceria of the right shape and size may prove to be the saving grace that will, if not solve, then alleviate the problem of antibiotic resistance in general and in-hospital infections in particular.

Safety and toxicity of nanoceria

The toxic properties of CeO_2-NPs are also associated with pro-oxidant properties, and as seen in the previous paragraph, they are manifested in acidic environments [24]. Data supporting caspase-dependent cell death are presented in Mittal & Pandey [62]. And also, the results of the study on mice are presented in a review by Rajeshkumar, where reactive oxygen species induced DNA lesion and cell cycle arrest, which caused apoptotic cell death [20]. The same review confirms the dependence of the effect on synthesis conditions and pH of the medium. In one study, a neurotoxic effect was observed in the formation of a complex with serotonin: 5-HT nanoceria, both in the brain and in the intestine (orally with prolonged exposure for more than 3 days) [63]. Meanwhile, in another study, ~10 nm nanoceria prolonged the lifespan and preserved neuronal function while protecting against aβ toxicity and ROS [64]. Considering the multi-enzymatic abilities of cerium depending on the acid-base state of the medium, it can be assumed that cell death is due to the 4-valency of cerium. This behavior of CeO_2-NPs in media with different acidity is the basis for explaining the differential cytotoxicity of the material in relation to tissues with different pH values [65].

In most studies, cytotoxicity increases with increasing levels of nanoceria used [62]. In general, viability of normal cells is maintained up to 10 mM (10^{-2} M); in some studies, the level is even higher compared to the control. In addition, selective toxicity is detected even at low cerium levels for malignant cells that have low pH values; this opens new solutions for targeting malignant tumors in oncology [34]. Some studies have shown that higher levels (up to 250 μg/mL) preserved more than 80% of viable cells [41]. However, there is evidence that bioaccumulation of CeO_2-NPs can cause genotoxic effects, which, however, requires confirmation in long-term studies [66, 67].

CONCLUSION

Thus, it can be concluded that there are great potential prospects for the use of medical products incorporating CeO_2-NPs in the regeneration of wounds of various origins.

Based on analysis of selected publications, we can draw the following initial conclusions about the results of research on nanoceria for early regeneration and the requirements for nanoparticles obtained in the process of synthesis:

1. The most common shape of nanoparticles is the spherical (octahedral) one due to high transcellular permeability or the cubic one. The latter has been drawing attention from researchers for its stability due to its edges and increased restorative capacity resulting from increased number of vacancies.
2. The size should be the smallest possible (<8 nm) with the smallest range. By doing so, a certain degree of stability in the observed effects is achieved. In addition, the size of the nanoparticles in the ash should not differ significantly from that obtained by positron emission tomography (PET) imaging (or other "dry" diameter measurement methods). In such a case, the nanoparticles do not aggregate into agglomerates, which also stabilizes their final in vitro and in vivo performance characteristics.
3. Nanoparticle coating should provide for additional properties that depend on the very nature of the shell. It may be increased stability or solubility. Besides,

the coating may also potentiate the antioxidant or antimicrobial effects of cerium oxide itself.

4. Synthesis method (chemical/physical/"green") should be chosen based on availability of raw materials, technical capabilities of the laboratory, simplicity and ability to repeat experiments so many times with the same result, thus satisfying all the requirements for drugs and/or medical products.
5. The dosage form should be accessible in terms of technology, taking into account mass production, and easy to use for the patient and medical staff without the need for additional training. Such forms are gel and transdermal systems (e.g., patch). However, when synthesizing them, it is necessary to select a material that is biocompatible, stable, able to support gas exchange, absorbs excess exudate, has mechanical protection and ideally antimicrobial-barrier, as well as a structure with matrix properties to facilitate cell migration and proliferation.
6. The pH of the environment (wound) and the dosage form should be taken into account to maximize regeneration.
7. The mechanisms by which CeO_2-NPs participate in regeneration are still debatable at the moment. However, the antioxidant effect of nanoceria (which starts at pH <5) is obviously proven. Antimicrobial effect (its presence and stability) is also discussed: the effect is not shown in all studies in relation to a narrow range of bacteria and is bacteriostatic in nature. There are evidence indicating stimulation of genes responsible for wound regeneration – angiogenic and growth factors.
8. Toxic effects are pro-oxidant in nature and presumably occur only in acidic environments.

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The authors declare no conflict of interest.

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