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Nanoceria-to-Curcumin Molar Ratio as a Critical Engineering Parameter for Biocompatible Redox-Active Nanoconjugates

Nikita N. Chukavin

chukavinnik@gmail.com

Institute of Theoretical and Experimental Biophysics of the Russian Academy of Sciences

Viktoriia A. Anikina

Institute of Theoretical and Experimental Biophysics of the Russian Academy of Sciences

Darya A. Vinnik

Institute of Theoretical and Experimental Biophysics of the Russian Academy of Sciences

Nikita A. Pivovarov

Institute of Theoretical and Experimental Biophysics of the Russian Academy of Sciences

Boris A. Bokl

Institute of Theoretical and Experimental Biophysics of the Russian Academy of Sciences

Anton L. Popov

Institute of Theoretical and Experimental Biophysics of the Russian Academy of Sciences

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Abstract

Curcumin is a natural polyphenol with broad therapeutic potential. However, its poor aqueous solubility and limited stability hinder its biomedical application. Cerium dioxide nanoparticles (nanoceria, CeO_2) have emerged as a promising platform for curcumin delivery and stabilization while simultaneously providing intrinsic redox activity. Here, we systematically investigated the nanoconjugates with nanoceria-to-curcumin molar ratios ranging from 10:1 to 100:1 to establish the relationship between nanoformulation composition and cellular response. Nanoformulations prepared at ratios of 10:1-20:1 contained excess unbound curcumin that induced significant cytotoxicity. Meanwhile, formulations with ratios of 25:1 and above showed no detectable cytotoxicity, consistent with the absence of excess unbound curcumin. Based on these findings, a molar ratio of 25:1 was identified as the optimal formulation, providing an optimal balance between nanoparticle surface loading and biological safety. The optimized nanoconjugate effectively protected human keratinocytes (HaCaT), mesenchymal stem cells (hMSc), and fibroblasts (HF) against hydrogen peroxide-induced oxidative stress, demonstrating preserved antioxidant activity without the adverse effects associated with excess unbound curcumin. Collectively, these findings identify nanoceria-to-curcumin ratio as a critical engineering parameter governing the safety and bioactivity of nanoceria-curcumin nanoformulations and provide a rational framework for their design and optimization.

1. Introduction

Curcumin is a natural hydrophobic polyphenol extracted from the rhizomes of Curcuma longa. It possesses a broad spectrum of confirmed biological activities [1–3]. Specifically, numerous studies demonstrated its anti-inflammatory properties, associated with the suppression of pro-inflammatory mediators [4–6]. The bioactivity of curcumin is largely determined by its molecular structure, which consists of two aromatic rings with hydroxyl and methoxy substituents connected by a linker of two α,β-unsaturated carbonyl groups. This configuration enables the existence of keto-enol tautomerism and a β-diketone form [7]. Owing to these structural features, curcumin can interact with and modulate various biomolecules, thereby influencing cellular signaling pathways [8, 9]. However, curcumin has poor aqueous solubility and limited stability, as well as decreased bioavailability and rapid metabolic clearance [10–13]. To address these drawbacks, several strategies have been proposed. One of them is the design of co-crystals, which improve physicochemical parameters and enhance stability [14]. Previously developed co-crystals of curcumin exhibited reduced melting points and significantly higher solubility [15–18]. Other approaches involve the use of nanocarriers, such as nanoparticles [19, 20], micelles [21–23], dendrimers [24], liposomes [25] and exosomes [26], which provide stabilization and facilitate delivery. Therefore, curcumin represents a biologically active compound with significant biomedical potential, especially when combined with stabilizing nanoformulations.

Cerium-based nanomaterials, or nanoceria, offer a suitable platform for curcumin conjugation. In particular, cerium dioxide nanoparticles (CeO_2 NPs) display enzyme-mimicking properties resembling those of superoxide dismutase (SOD) [27–29], catalase (CAT) [30], peroxidase (POD) [31], oxidase (OXD)

[32], nuclease [33], phospholipase [34] and photolyase [35]. Their catalytic activity originates from the peculiarities of the crystal lattice, coexistence of Ce^{3+}/Ce^{4+} ions, and the presence of oxygen vacancies. These parameters, together with particle configuration and environmental conditions, define the biological behavior of nanoceria [36–38]. Importantly, nanoceria are able to exert anti-inflammatory effects through the downregulation of pro-inflammatory mediators [39, 40]. Another cerium-based material, cerium fluoride nanoparticles (CeF_3 NPs), also exhibits redox activity. Due to the mechanism they are able to selectively modulate cellular response to X-ray [41] and proton beam irradiation [42]. CeF_3 NPs can also be modified with various ligands and elements for photodynamic therapy (PDT) [43] and luminescent imaging [44]. Moreover, both CeO_2 and CeF_3 NPs have demonstrated radioprotective effects in Schmidtea mediterranea exposed to X-rays [45] and synergistic antimicrobial activity when combined with cold atmospheric plasma [46]. The abovementioned features highlight nanoceria as a promising basis for biomedical applications.

Surface modification of cerium dioxide nanoparticles enables the generation of hybrid nanoformulations with novel or enhanced functionalities. A wide range of nanoceria -based systems has been described, including conjugates with FITC [47], ssDNA [48], labeled dsDNA [49], miRNA-146a [50–52], doxorubicin [53], doxorubicin and folate [54], clindamycin [55], pilocarpine, chitosan and ZM241385 [56], TNF-α [57], morin [58], as well as gadolinium-doped CeO_2 NPs [59]. For instance, a CeO_2-mildronate composite displayed mitochondria-targeted radioprotective effects [60]. Another nanoconjugate of nanoceria and calcein not only neutralizes reactive oxygen species (ROS) but also allows monitoring of intracellular ROS levels [61, 62].

In recent years, particular attention has been given to the development of nanoceria–curcumin formulations. The interaction between curcumin and nanoceria is mediated mainly through hydrogen bonding and chelation [63]. Various strategies have been proposed, including colloidal nanoconjugation [64], encapsulation into copolymers [65], incorporation into extracellular matrix [66], hydrogels [67] and biopolymers [68], stabilization by polysaccharides [69], as well as binding through phospholipids [70] and coordination with polypeptides [71]. These nanosystems display a range of beneficial biological activities such as anti-inflammatory, antioxidant, wound-healing, and antitumor effects, opening perspectives for their use in cancer therapy and chronic inflammatory diseases. Meanwhile, effect of nanoceria-to-curcumin ratio in the above mentioned nanoformulations remains poorly understood. This aspect requires a more detailed study, since at certain ratios of nanoceria -curcumin may demonstrate a cytotoxic effect. Previously, we showed that nanoceria-curcumin nanoconjugate with a ratio of 10 to 1 contains excess curcumin, which causes cytotoxicity of this nanoconjugate [72]. At the same time, nanoceria act as stabilizer and carrier of curcumin, increasing its biocompatibility. The objective of this work is to investigate how different ratios of nanoceria -curcumin affect safety and bioactivity of respective nanoconjugate. In particular, we found an optimal nanoceria-to-curcumin ratio, at which excess curcumin is absent, and investigated the antioxidant activity of respective nanoconjugate in a model of oxidative stress induced by hydrogen peroxide.

2. Materials and Methods

2.1. Reagents and synthesis

For the preparation of the nanoceria-curcumin nanoconjugate, chemically pure cerium ammonium nitrate ($(NH_4)_2Ce(NO_3)_6$), curcumin, polyvinylpyrrolidone (PVP), 96% ethanol (Sigma-Alrdich, USA) and deionized water were used. Cell viability and death tests were conducted with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide, PanEco, Russia), Hoechst 33342 and propidium iodide dyes (Lumiprobe, Russia).

Cerium dioxide nanoparticles were synthesized following the procedure reported by Shcherbakov et al. [73]. The CeO_2-curcumin nanoconjugate was then prepared according to the approach described by Zholobak and co-authors [64], which forms the basis of this study. Briefly, the obtained nanoceria colloidal solution was mixed with PVP in water at a 1:1 weight ratio. The resulting nanoceria –PVP sol was dried in an ED 115 heating chamber (Binder, Germany) at 100°C. Separately, a curcumin–PVP solution was prepared in 96% ethanol at a mass ratio of 1:10. The dried nanoceria –PVP was redispersed in 96% ethanol and combined with the curcumin–PVP solution at molar ratios of nanoceria -curcumin from 10:1 to 100:1. The resulting samples of nanoceria –curcumin colloidal solutions were dried at 50°C and finally redispersed in deionized water. Samples of nanoceria and curcumin, stabilized with PVP, were obtained in the same way (Fig. 1a).

2.2. Physico-chemical properties

Absorption spectra of the samples were recorded using a Cary 5000 spectrophotometer (Agilent Technologies Inc., USA) over the range 340–640 nm. Measurements were performed in quartz cuvettes with deionized water as a reference. Absorption spectrum of nanoceria was subtracted from the absorption spectra of all nanoceria -curcumin samples. Then the optical absorption spectra of the nanoconjugate with ratios ranging from 1:15 to 1:100 were recalculated relative to that of the nanoconjugate with a ratio of 10:1. Morphology of nanoceria -curcumin, nanoceria and curcumin nanoparticles was examined by transmission electron microscopy using a JEM-2010 (JEOL, Japan) transmission electron microscope at acceleration voltage of 200 kV. Additionally, morphology of nanoceria -curcumin was analyzed by scanning electron microscopy using a NVision 40 (Carl Zeiss, Germany) scanning electron microscope at 30 keV. Hydrodynamic size and zeta potential were determined by dynamic and electrophoretic light scattering (DLS and ELS, respectively) in deionized water, cell culture media DMEM with a high glucose content and DMEM/F12 (1:1) both with an addition of 50 μg/mL streptomycin, 50 μg/mL penicillin, 2 mM L-glutamine (GPS) and 10% fetal bovine serum (FBS) (PanEco, Russia) at 25°C using a BeNano 90 Zeta analyzer (Bettersize, China).

2.3. Cell culture

Experiments were performed with immortalized human keratinocytes (HaCaT), primary fibroblasts (HF) as well as primary mesenchymal stem cells (hMSc), obtained from the cryobank of the Laboratory of

Theranostics and Nuclear Medicine of the Institute of Theoretical and Experimental Biophysics of the Russian Academy of Sciences (ITEB RAS, http://nanomedlab.ru/). These cell models represent skin, connective tissue and mucosal cells, which are relevant targets for nanoceria-curcumin nanoformulations. The cells were cultured using DMEM medium with a high glucose content (for HaCaT) and DMEM/F12 (1:1) (for HF and hMSc) with an addition of GPS and 10% FBS. Cells were seeded in flasks with 25 or 75 cm^2 growth areas and in 96-well plates (SPL Life Sciences, Korea). Cell cultures were incubated in a D180 CO_2 incubator (RWD Life Science, China) at 37°C, 5% CO_2, and 95% humidity. All manipulations with cells were carried out under a laminar flow using a Neoteric BMB-II-Laminar flow cabinet-1.2 (LamSystems, Russia). Samples were added 24 hours after cell seeding.

2.4. Cell viability

Cell viability after exposure to the studied samples was evaluated by MTT assay. After 24, 48 and 72 hours of cells co-incubation with the nanoceria -curcumin at different component ratios (from 10:1 to 100:1), cell culture medium was replaced with serum-free medium containing MTT at 0.5 mg/mL. After 3 hours incubation, the MTT solution was substituted with pure DMSO (Component-Reactive, Russia), and the plates were shaken for 10 min. Absorbance was then measured at 570 nm using an INNO-S multimode plate reader (LTek, Korea). The optical density reflects the amount of formazan produced by intracellular NADPH-dependent oxidoreductases, which correlates with the cell viability of metabolically active cells. Data were normalized to untreated control cells and expressed as mean values ± standard deviation (SD). Biological replicates were n = 5. Statistical significance between treated and control groups was assessed with Welch's t-test at significance thresholds of $0.01 < p < 0.05$ (*), $0.001 < p < 0.01$ (**), $0.0001 < p < 0.001$ (***) and $p < 0.0001$ (****), using GraphPad Prism software.

2.5. Cell death

Cell death was analyzed by Live/Dead staining protocol. After 24, 48 and 72 hours of cells co-incubation with the nanoceria -curcumin at different component ratios (from 10:1 to 100:1), cell culture medium was replaced with Hank's Balanced Salt Solution (HBSS, PanEco, Russia) containing Hoechst 33342 (DNA stain for all cells, Ex = 350 nm, Em = 460 nm) and propidium iodide (DNA stain for membrane-compromised cells, Ex = 535 nm, Em = 615 nm). After 15 min incubation, cells were washed once with HBSS and imaged using a ZOE fluorescence microscope (Bio-Rad, USA). ImageJ software was employed to count total and dead cells, and the ratio was calculated. Cytotoxicity was quantified as IC_{50}, defined as the concentration at which 50% of the cells were non-viable.

2.6. Antioxidant activity

Antioxidant activity of the CeO_2-curcumin nanoconjugate was investigated in a model of oxidative stress, induced by hydrogen peroxide. The analysis was performed using the nanoceria-curcumin (25:1 molar ratio) at concentrations from 10 to 500 μM, nanoceria at a concentration of 500 μM and curcumin at a concentration of 25 μM. Two approaches of samples addition were used: preventive, at which the samples were added to the cells for overnight co-incubation before the oxidative stress induction (Fig. 3a), and therapeutic, at which the samples were added to the cells after the oxidative stress

induction (Fig. 4a). The oxidative stress model was induced by one-hour co-incubation of the cells with hydrogen peroxide (Sigma-Aldrich, USA), dissolved in HBSS at concentrations of 200 and 500 μM. After the oxidative stress was induced, the H_2O_2 solution was replaced with cell culture medium (with or without samples), then cells were cultured for 24 hours. Next, cell viability was measured as described previously.

3. Results

3.1. Nanoceria-to-curcumin ratio affects cytotoxicity of their nanoconjugate

We synthesized the CeO_2-curcumin nanoconjugate with molar ratios of the components ranging from 10:1 to 100:1 through a multi-step scheme (Fig. 1a), which we used in our previous work [72]. The first confirmation of the nanoconjugate formation was a change in the shade of its colloidal solution in relation to CeO_2 NPs and curcumin alone: when mixing a suspension of CeO_2 NPs (pale yellow shade) and a solution of curcumin (light orange shade), an nanoceria -curcumin sol of a dark orange shade was formed (Fig. 1b). These changes are the most noticeable when comparing the samples of CeO_2 NPs and curcumin with the nanoconjugate sample with a component ratio of 10:1, which has respective concentration of the components. Even at a ratio of 100:1, which has the lowest curcumin content, the effect of shade change was observed. Additionally, a comparative analysis of optical absorption spectra of curcumin alone and in the composition of nanoconjugate samples showed a broadening of the curcumin absorption peak into the long-wavelength region (Fig. 1c). The broadening indicates changes in the electronic configuration of curcumin upon binding to nanoceria, thus confirming the formation of CeO_2-curcumin nanoconjugate. The effects revealed reproduce the data we previously obtained on the CeO_2-curcumin nanoconjugate with a ratio of 10:1 [72].

Next, we investigated a presence of excess curcumin in the nanoconjugate samples obtained. First of all, we revealed a hypsochromic shift of the curcumin optical absorption peak with the increasing nanoceria-to-curcumin ratio (Fig. 1c). Specifically, the peak gradually shifts from 417 nm to the short-wavelength region at ratios from 10:1 to 20:1, reaching a final value of 387 nm at a ratio of 25:1. This effect indicates not only a change in the electronic configuration of curcumin due to its energetically beneficial process of binding to nanoceria [74–76], but also a decrease in the proportion of excess curcumin with an increase in the nanoceria-to-curcumin ratio. We have previously shown that a nanoconjugate with a ratio of 10:1 contains a nanoscale fraction of excess colloidal curcumin [72]. We suggest that this fraction causes the peak of absorption of curcumin, which is part of the nanoconjugate with a ratio of 10:1, to be found in a longer wavelength region corresponding to the peak of absorption of the curcumin alone (413 nm). In other words, starting from a ratio of 25:1 and above, there is no excess curcumin in the nanoconjugate samples.

To further prove the abovementioned statement, we analyzed the cytotoxicity of nanoconjugate samples with ratios ranging from 10:1 to 100:1. Previously, the nanoconjugate with a ratio of 10:1 demonstrated statistically significant cytotoxicity towards human immortalized keratinocytes (HaCaT), primary fibroblasts (HF) and mesenchymal stem cells (hMSc), caused by excess curcumin in its composition [72]. The hMSc cells proved to be the most sensitive to the cytotoxic effect of excess curcumin. In the current work, we applied the nanoceria-curcumin samples to the hMSc cells at a concentration of 500 μM (by nanoceria), which was the highest of those we had previously studied on the 10:1 ratio. A statistically significant decrease in the viability of these cells was revealed during co-incubation for 72 hours with the nanoconjugate samples with ratios ranging from 10:1 to 20:1 (Fig. 1d). The most pronounced effects were observed with a reduction in the ratio from 15:1 to 10:1. Meanwhile, the nanoceria-curcumin samples with the component ratios ranging from 25:1 to 100:1 exhibited no statistically significant cytotoxic effect. Importantly, we attribute the cytotoxic effect to excess curcumin, not to curcumin itself. For instance, the cytotoxicity of the nanoconjugate with ratios ranging from 20:1 to 10:1 increases non-linearly. Furthermore, if we correlate the concentration of curcumin in the nanoconjugate samples, then the curcumin content in the nanoconjugate (10:1) at a concentration of 200 μM corresponds to its content in the nanoconjugate (25:1) at a concentration of 500 μM. However, at the nanoconjugate (10:1) concentration of 200 μM, a decrease in the viability of hMSc cells was previously observed by more than 50% after 72 hours of co-incubation (IC50 = 211 μM) [72]. It is also worth noting that only in the presence of the nanoconjugate with a 10:1 ratio there was an increase in the frequency of cell death. We assume that the effect is caused by an excess of curcumin, significantly exceeding that in the nanoconjugate samples with ratios of 15:1 and 20:1. To summarize, the results obtained complement the spectrophotometry data, further confirming the absence of excess curcumin in the nanoceria-curcumin samples with a ratio of 25:1 or higher.

3.2. Optimal nanoceria-to-curcumin ratio of 25:1 ensures their exhaustive binding

Since the 25:1 ratio is the boundary between excess curcumin and incomplete loading of nanoparticles in the nanoconjugate, we chose it as the optimal one. Hereafter, the CeO_2-curcumin nanoconjugate with a molar ratio of the components of 25:1 will be designated as nanoceria-curcumin. In order to additionally prove the absence of excess curcumin and the complete formation of nanoconjugate, we conducted an analysis of the nanoceria-curcumin physico-chemical properties. According to the TEM and SEM data, the nanoceria-curcumin lacks of a nanoscale fraction of excess colloidal curcumin (Fig. 2a, Fig. S1), which was presented in the nanoconjugate with a ratio of 10:1 [72]. Combined with the results of UV- visible spectrophotometry and cytotoxicity analysis, this shows the absence of excess curcumin in the nanoceria-curcumin sample. Meanwhile, the binding of curcumin to nanoceria resulted in the hydrodynamic size increase from 25 to 26.3 nm (Fig. 2b) and zeta potential inversion from + 25 to -12.7 mV (Fig. 2c), regarding the respective parameters of nanoceria alone [72]. Similar changes were observed in the nanoconjugate sample with a component ratio of 10:1. However, the hydrodynamic size of the nanoconjugate (10:1) was larger by 3.7 nm, which may be due to the presence of excess curcumin

in the sample. Taking into account the results obtained, we can confidently state that the nanoconjugate, with the optimal nanoceria-to-curcumin ratio of 25:1, is devoid of excess curcumin and forms a stable nanoformulation of CeO_2-curcumin, ensuring the exhaustive binding of the components.

Before conducting a study of the nanoceria-curcumin antioxidant activity on cells, an analysis of its colloidal stability in cell culture media was carried out. It has been shown that the nanoceria-curcumin has increased in size by a factor of two (from 26.3 to 53 nm), when redispersed in DMEM and DMEM/F12 (1:1), both containing GPS and 10% FBS (Fig. 2d). We assume that the nanoconjugate was opsonized by components of the cell culture media and formed aggregates, but it still remained colloidally stable. Thus, the nanoceria-curcumin can be studied for its antioxidant activity under H_2O_2-induced oxidative stress *in vitro*.

3.3. Nanoconjugate with the optimal nanoceria-to-curcumin ratio shows antioxidant activity

To evaluate an antioxidant activity of the nanoceria-curcumin, we used two approaches: addition of the nanoconjugate before the induction of oxidative stress by hydrogen peroxide (mentioned as preventive action, Fig. 3a) and after it (mentioned as therapeutic action, Fig. 4a). Under the oxidative stress induced by 500 μM of H_2O_2, statistically significant preventive action of the nanoceria-curcumin was revealed on HaCaT and HF cells (Fig. 3b). Viability of the cells elevated with increasing nanoceria-curcumin concentration, reaching values at a concentration of 250 μM, which correspond to values obtained for nanoceria alone at a concentration of 500 μM. Furthermore, the addition of nanoceria-curcumin at a concentration of 500 μM caused the greatest antioxidant effect, overcoming the impacts of both 250 μM nanoceria-curcumin and 500 μM CeO_2 NPs alone. Taking into account the absence of antioxidant activity of free curcumin, we assume that the enhanced antioxidant activity of the nanoceria-curcumin is due to the action of curcumin, which was stabilized and delivered by nanoceria. This is consistent with the data we obtained earlier, according to which nanoceria provide increased biocompatibility and bioavailability of curcumin [72]. It should be noted, that hMSc cells were found to be the most sensitive to the oxidative stress, induced by 500 μM H_2O_2. Their viability decreased dramatically, and no one sample influenced it.

Concurrently, under the oxidative stress, induced by 200 μM of H_2O_2, the enhanced antioxidant activity of the nanoceria-curcumin was not observed (Fig. 3c). Although the nanoceria-curcumin showed antioxidant activity on HaCaT cells, this effect did not differ from that of free curcumin, while nanoceria alone failed to protect the cells from the oxidative stress. The data obtained on HF and hMSc cells also does not allow us to unequivocally assert the influence of nanoceria-curcumin antioxidant activity under these conditions. We propose that such a difference in the observed preventive action is due to both the conditions of oxidative stress induction, cellular uptake efficiency and the sensitivity of cell lines to it. Consequently, to analyze the antioxidant activity of nanoformulations, the parameters should be selected carefully for more precise and representative results.

During the analysis of the therapeutic action of nanoparticles in the model of oxidative stress, it was revealed that exposure to H_2O_2 at concentrations of 500 and 200 μM leads to a complete suppression of the viability of HaCaT and hMSc cells, regardless of the presence of samples (Fig. 4b and 4c). Therefore, both the conditions of oxidative stress induction and the cell lines are not suitable for this study. HF cells were found to be the most resistant to the cytotoxic effect of H_2O_2. However, both the nanoceria-curcumin and nanoceria alone did not protect the cells from the oxidative stress, induced by 500 μM (Fig. 4b). Interestingly, that free curcumin caused a decrease in the viability of HF cells under these conditions. The same effect was observed under the oxidative stress, induced by hydrogen peroxide at a concentration of 200 μM (Fig. 4c). Nevertheless, the nanoceria-curcumin showed its antioxidant activity, along with nanoceria alone, under these conditions. This is consistent with the results obtained in the «preventive action» scheme using 500 μM H_2O_2 on HF cells (Fig. 3b). Thus, the nanoconjugate with the optimal nanoceria-to-curcumin ratio shows antioxidant activity.

4. Discussion

We further analyzed and reproduced the previously developed nanoconjugate synthesis method using a new range of their molar ratios from 10:1 to 100:1. The optical absorption of curcumin has undergone significant changes, resulting in a long-wavelength broadening and a hypsochromic shift. Indeed, such changes in the optical properties of molecules may indicate a transformation of their electronic configuration during energy-beneficial processes of spontaneous interaction [74–76]. Furthermore, the identified effects are consistent with the data we obtained earlier and indicate the formation of a stable nanoconjugate structure [72]. This also aligns with the findings of previous studies on the ability of nanoceria to form stable nanoformulations with curcumin [63–65, 69–71]. Meanwhile, the ratios of nanoceria-to-curcumin from 10:1 to 20:1 caused the formation of a nanoconjugate with an excess of cytotoxic curcumin, while ratios from 25:1 and above were devoid of this component. This highlights that selection of an optimal ratio of components of biologically active nanoformulations such as nanoceria-curcumin is a necessary step for their development and further clinical applications.

The nanoceria-to-curcumin ratio of 25:1 serves as the threshold between the excess of curcumin and the insufficient loading of nanoceria in the nanoconjugate. Consequently, we have proposed this ratio as the optimal one. Based on our findings, we can assert with certainty that the nanoconjugate with the optimal ratio of 25:1 is free from excess curcumin and forms a stable hybrid nanoformulation of nanoceria and curcumin, guaranteeing exhaustive binding of these components. This particular configuration of the nanoconjugate also exhibits colloidal stability in cell culture media (DMEM and DMEM/F12, both containing 10% FBS), making it suitable for testing its biological activity in a variety of cellular models in vitro. These may include cellular models of oxidative stress, inflammation, radiation-induced injury, tumors and wounds.

In this work, we experimentally confirmed that the CeO_2-curcumin nanoconjugate has pronounced antioxidant activity under H_2O_2-induced oxidative stress. Depending on the conditions of the

experimental model (scheme of the nanoconjugate addition and the concentration of H_2O_2) and the cell lines used (HaCaT, HF and hMSc), the antioxidant effect was shown to be enhanced by simultaneous action of nanoceria and curcumin or be the same as the free components action. In previous studies, both nanoceria and curcumin showed antioxidant activity [77–80]. We propose that nanoceria may ensure the antioxidant activity of curcumin by its stabilization and delivery. However, under certain conditions, no antioxidant activity of the nanoconjugate was detected, which underscores the importance of selecting and validating the cell models used. In particular, dose of inductor, period of induction, scheme of nanoformulation addition, as well as co-incubation time after induction are significant parameters whose rational modulation can provide the most accurate data for convincing conclusions.

Thus, nanoceria is able to bind curcumin on its surface, forming stable hybrid nanoconjugates. Such nanoformulations may provide enhanced delivery of curcumin. However, depending on the nanoceria-to-curcumin ratio, these nanoformulations may retain excess curcumin, which could contribute to undesirable cytotoxic effects. Selecting an optimal ratio makes it possible not only to reduce cytotoxicity, but also to enhance the nanoconjugate antioxidant activity. Considering all of the above, optimizing the synthesis strategy for curcumin-based nanoformulations with nanoceria to minimize the presence of unbound curcumin is a critical step toward improving their biomedical safety and effectiveness. We propose that any residual free curcumin could significantly restrict the therapeutic dosage of any potential nanoformulations. Further studies are recommended to assess a potential bioactivity of the respective nanoformulation with optimal nanoceria-to-curcumin ratio in various cellular and animal models. Such investigations may reveal enhanced antioxidant, anti-inflammatory, anti-tumor or wound-healing activities of the nanoconjugate.

Declarations

Competing Interests:

Authors declare no conflict of interests.

Informed Consent

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Author Contribution

Conceptualization – N.N.C. and A.L.P.; methodology, validation – V.A.A., D.A.V., N.A.P., B.A.B.; investigation – N.A.C., V.A.A., D.A.V., N.A.P., B.A.B.; resources – A.L.P.; data curation, formal analysis, software, visualization, writing (original draft preparation) – N.N.C.; supervision, writing (review and editing) – A.L.P.; project administration and funding acquisition – A.L.P. All authors have given approval to the final version of the manuscript.

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Data Availability

Data is contained within the article.

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Figures

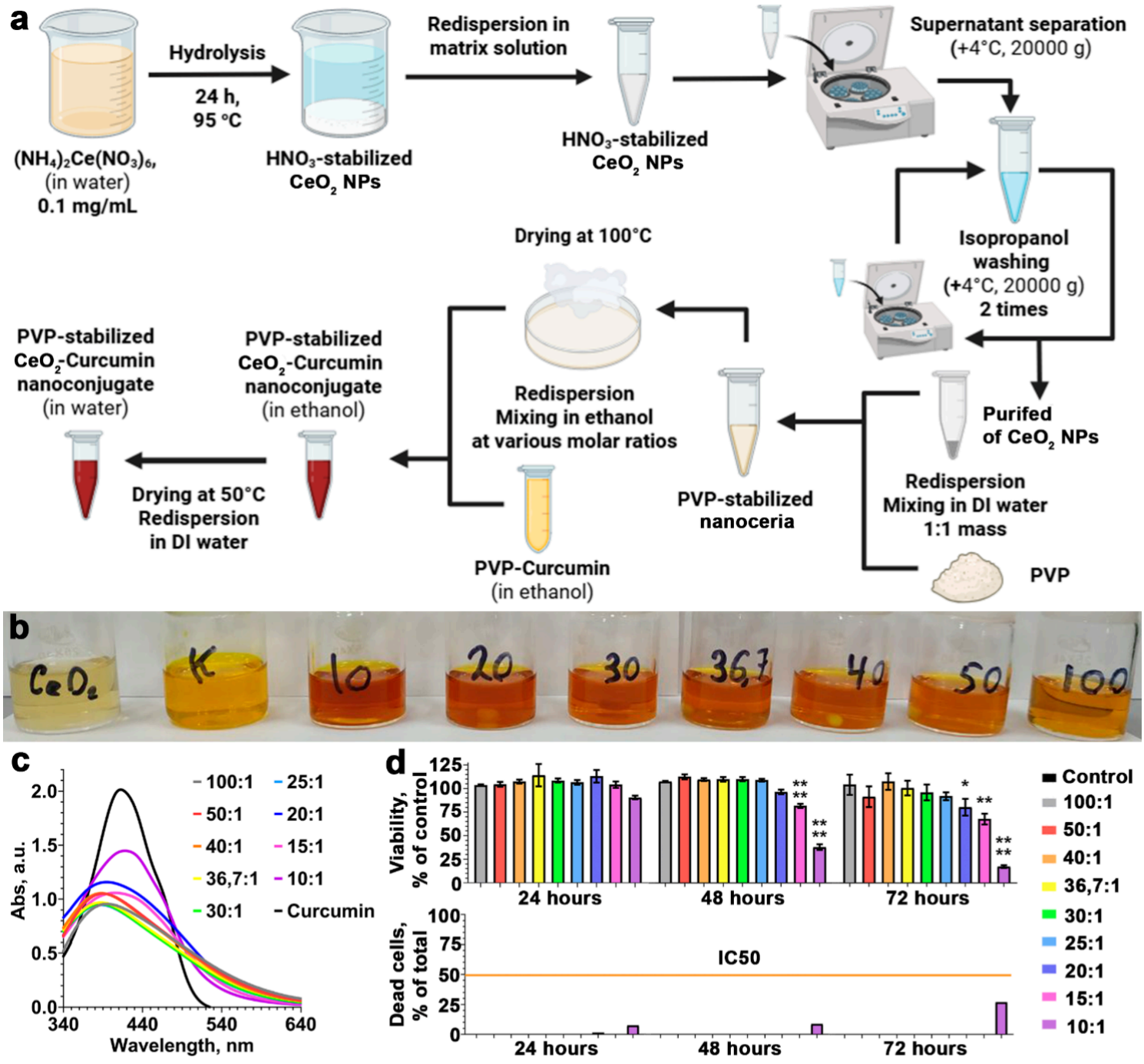

Figure 1

Synthesis scheme of CeO_2-curcumin nanoconjugate (a). From left to right: samples of CeO_2 NPs, curcumin and CeO_2-curcumin nanoconjugate with molar ratios of the components ranging from 10:1 to 100:1. Concentrations of nanoceria and curcumin alone correspond to their concentrations in the nanoconjugate with a ratio of 10:1 (b). Optical absorption spectra of curcumin alone and in the composition of nanoconjugate with molar ratios of the components ranging from 10:1 to 100:1 (c). From top to bottom: viability and death frequency of hMSc after 24, 48, and 72 hours of co-incubation with CeO_2-curcumin nanoconjugate with molar ratios of the components ranging from 10:1 to 100:1 at a concentration of 500 μM (by nanoceria) (d)

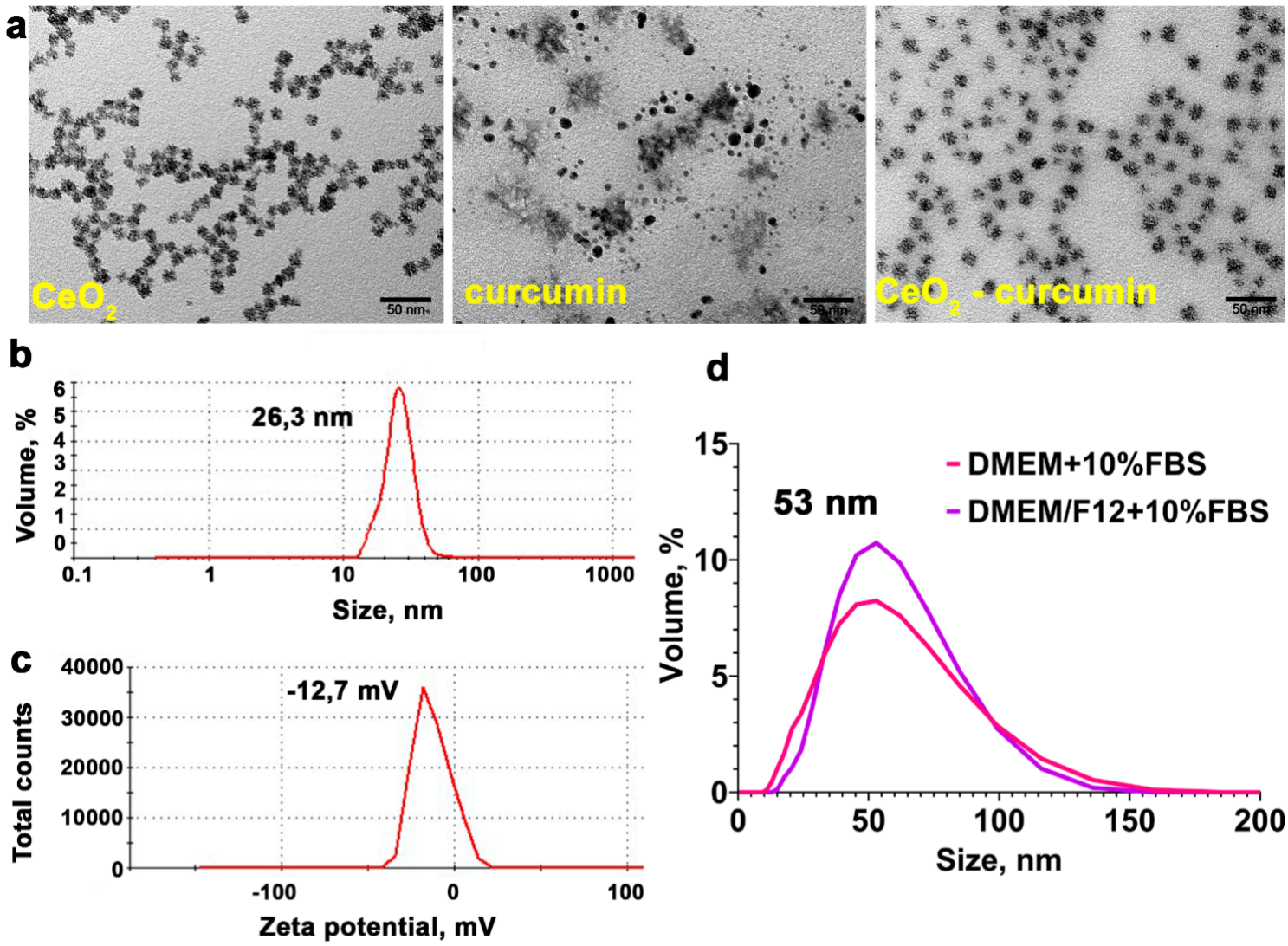

Figure 2

(a) TEM images of the CeO_2-curcumin nanoconjugate with a molar ratio of the components of 25:1 (further designated as nanoceria-curcumin), nanoceria and curcumin alone. Scale bars are equal to 50 nm. Hydrodynamic size (b) and zeta potential (c) distributions of the nanoceria-curcumin redispersed in deionized water. (d) Distribution of hydrodynamic size of the nanoceria-curcumin redispersed in DMEM and DMEM/F12 (1:1) cell culture media, which both are containing GPS and 10% FBS SEM image of the nanoceria-curcumin

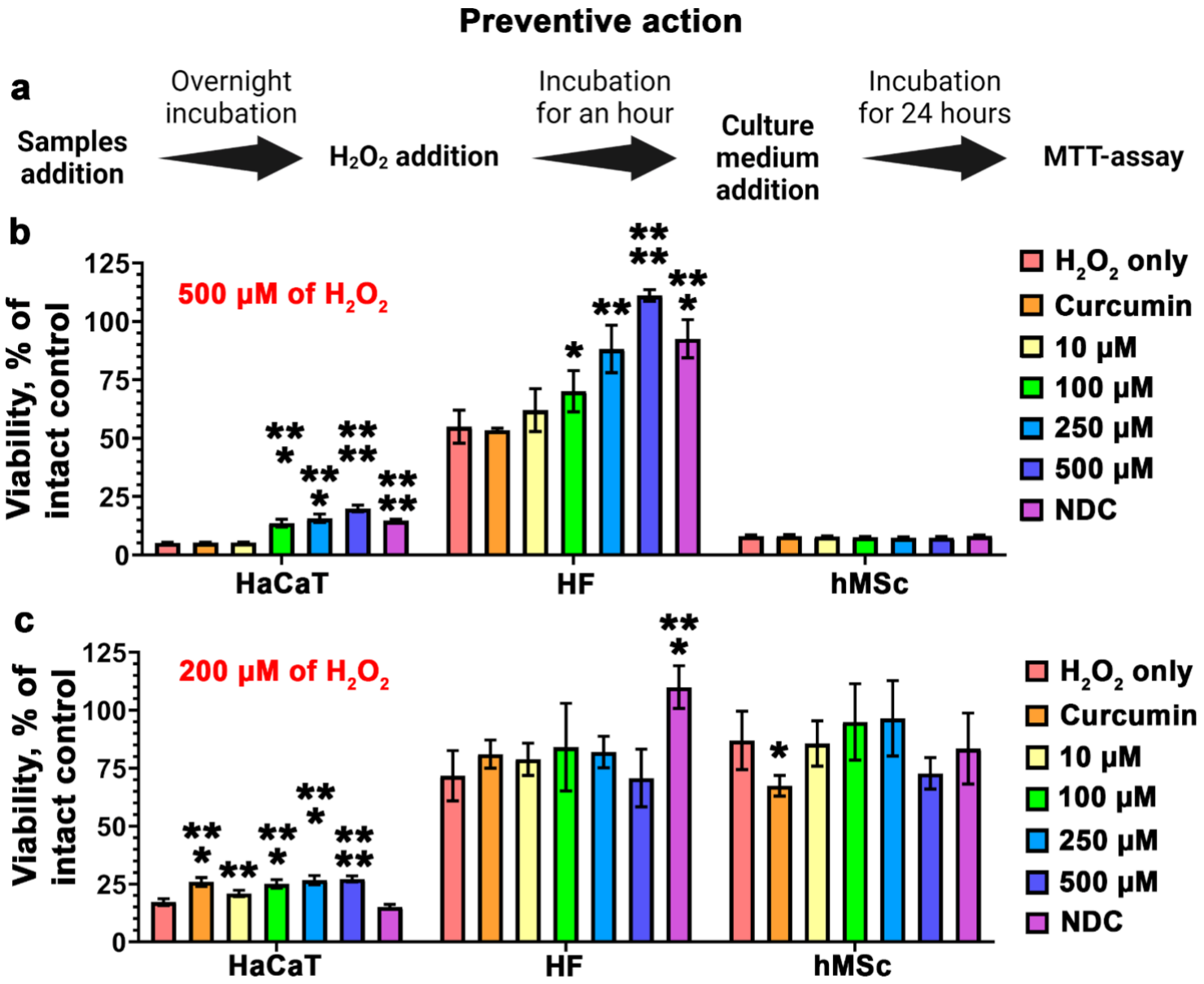

Figure 3

Scheme of the analysis of antioxidant activity of the CeO_2-curcumin nanoconjugate with a molar ratio of the components of 25:1 with its preliminary addition (a). Viability of HaCaT, HF and mesenchymal hMSc 24 hours after oxidative stress model induction by H_2O_2 at concentrations of 500 μM (b) and 200 μM (c). Before the induction, the cells were co-incubated overnight with the nanoceria-curcumin (10–500 μM by nanoceria), nanoceria (500 μM) and curcumin (25 μM) alone. The percentage values of the intact control are given as Mean ± SD, n=5. The statistical significance of the differences between the H_2O_2 only treated group and other experimental groups was confirmed using the Welch t-test, where * at $p<0.05$, ** at $p<0.01$, *** at $p<0.001$, and **** at $p<0.0001$

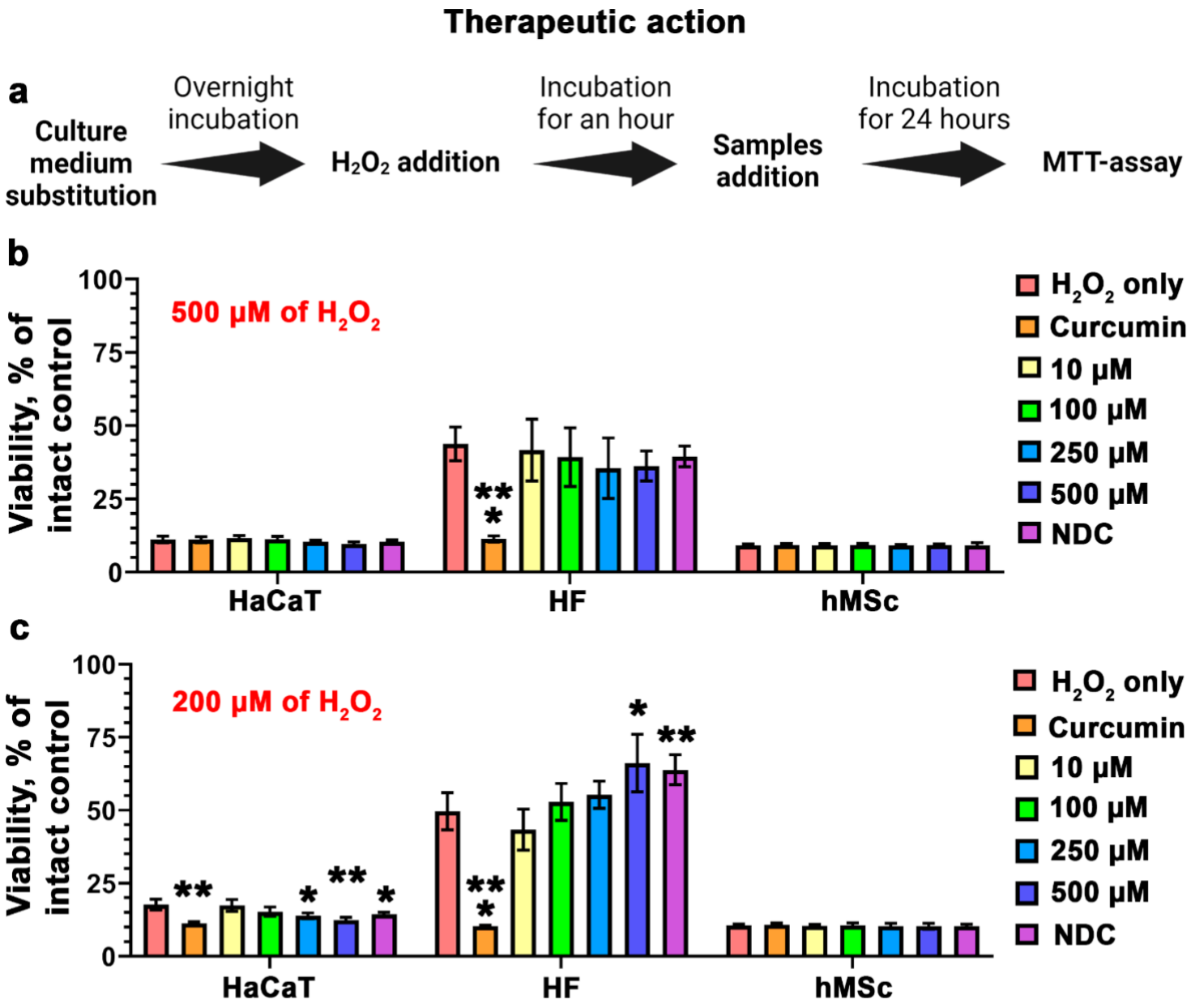

Figure 4

Scheme of the analysis of antioxidant activity of the CeO_2-curcumin nanoconjugate with a molar ratio of the components of 25:1 with its addition after oxidative stress induction (a). Viability of HaCaT, HF and hMSc 24 hours after oxidative stress model induction by H_2O_2 at concentrations of 500 μM (b) and 200 μM (c). After the induction, the cells were co-incubated with the nanoceria-curcumin (10–500 μM by nanoceria), nanoceria (500 μM) and curcumin (25 μM) alone for 24 hours. The percentage values of the intact control are given as Mean ± SD, n=5. The statistical significance of the differences between the H_2O_2 only treated group and other experimental groups was confirmed using the Welch t-test, where * at $p<0.05$, ** at $p<0.01$, *** at $p<0.001$, and **** at $p<0.0001$

Supplementary Files

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- Supplementary.docx
- floatimage1.png