Antiviral efficacy of cerium oxide nanoparticles

Alexandra Nefedova

University of Tartu

Kai Rausalu

University of Tartu

Eva Zusinaite

University of Tartu

Alexander Vanetsev

University of Tartu

Merilin Rosenberg

University of Tartu

Kairi Koppel

University of Tartu

Stevin Lilla

University of Tartu

Meeri Visnapuu

University of Tartu

Krišjānis Šmits

University of Latvia

Vambola Kisand

University of Tartu

Tanel Tätte

University of Tartu

Angela Ivask (✉ angela.ivask@ut.ee)

University of Tartu

Research Article

Keywords: Antiviral, virucidal, bactericidal, CeO₂, SiO₂, silver nanoparticles

Posted Date: February 25th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1382761/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Nanomaterials have been proposed as good candidates for the elimination of viruses due to their multimodal mechanisms of action. Here, we tested the antiviral potential of cerium dioxide (CeO_2) nanoparticles which is largely unexplored.

Two types of nano- CeO_2 particles with opposing surface charge, nano- $\text{CeO}_2(+)$ and nano- $\text{CeO}_2(-)$, were assessed for their capability to decrease the plaque forming units (PFU) of four enveloped and two non-enveloped viruses during 1 h exposure. Statistically significant antiviral activity of nano- CeO_2 towards enveloped coronavirus SARS-CoV-2 and influenza virus A/WSN/1933 was registered already at 20 mg Ce/l. Significant decrease in PFU of other two enveloped viruses, transmissible gastroenteritis virus TGEV and bacteriophage $\phi 6$ was evidenced at 200 mg Ce/l. For all the enveloped viruses the maximum reduction of PFU after 1 h exposure to nano- CeO_2 exceeded 2 logs, that has been considered as the lowest biologically meaningful activity in antiviral applications. For most of the enveloped viruses, 1 h exposure to nano- CeO_2 resulted in ≥ 4 log reduction in PFU. As expected, the sensitivity of non-enveloped viruses towards nano- CeO_2 was significantly lower than that of enveloped viruses. Until the highest tested concentration, 2000 mg Ce/l neither of the nano- CeO_2 showed an effect on picornavirus EMCV and only nano- $\text{CeO}_2(-)$ caused a slight non-monotonic response in MS2 bacteriophage.

Parallel testing of antiviral activity of Ce^{3+} ions and SiO_2 nanoparticles allows to conclude that nano- CeO_2 activity was due to neither released Ce^{3+} ions nor nonspecific effects of any nanosized particulate. Interestingly, we did not evidence any significant antiviral activity of Ag nanoparticles at 1 h exposure. This result referred to notably higher antiviral activity of nano- CeO_2 compared with nanosilver that has been generally considered as active against viruses. Although exhibiting antiviral effects, the antibacterial activity of nano- CeO_2 was very low. These results along with non-existent cytotoxicity nano- CeO_2 allow us to propose nano- CeO_2 for specific antiviral applications.

1. Background

The search for antiviral agents – materials that enable to inactivate viruses, inhibit their capability to infect their host cells or suppress their ability to replicate [1], has clearly intensified with the current COVID-19 pandemic [2]. Recently, the potential of biopolymer technology and nanotechnology in the development of antiviral therapeutics has been acknowledged [3–7] and numerous papers on nanomaterial-based inhibitors of viral replication cycle [4] (and references therein) or nanoparticles as drug delivery systems in targeted therapy [8] have been published. One of the groups of potential antiviral nanomaterials is metal and metal oxide nanoparticles [9] that have been suggested to exert their activity via multimodal mechanisms of action [10], including direct binding to virus surface, inhibiting viral binding to host cells or even interacting with viral genome [11]. Such a broad spectrum of proposed antiviral activities of metal-based nanoparticles may result in a smaller likelihood of developing antiviral resistance, which may occur in case of conventional antiviral drugs [12].

A wealth of literature has already been published on antiviral nanoparticles. As of January 2022, 1,623 articles were retrieved from ISI Web of Science using keywords “antiviral” AND “nanoparticle*”. Out of those 17% mentioned “COVID” while 30% included “silver”, 5% “copper”, 5% “zinc” and 4% “titanium OR titania”. Interestingly, all those nanoparticles are also among the most utilized nanoparticles in antibacterial applications [13] indicating that a relatively general mechanism of action, effective against both, bacteria and viruses, may be expected. Nanosilver contributing to 1/3 articles on antiviral nanoparticles, is clearly one of the most studied antiviral nanoparticle types. Potential binding of nanosilver particles onto the outer surface of the viruses and binding of nanoparticles to viral genetic material, leading to further inhibition of virus replication, have been suggested as its modes of action [14]. Efficacy of silver nanoparticles in decreasing the infective viral counts has been demonstrated against a variety of viruses, including HIV [15–18], herpes simplex virus [19], influenza virus [20], noroviruses [21] adenoviruses as well as SARS -CoV-2 [22] and other viruses [23–26]. It is however worth mentioning that while the antiviral concentrations of silver nanoparticles usually range between tens and hundreds of mg/l [27], those concentrations may already lead to cytotoxicity [22] and certainly exhibit antibacterial effect, that usually is evidenced starting from low mg/l range [28]. Indeed, unspecific cytotoxicity and concurrent potential health hazard of some of the proposed nanoparticles may be an issue [29] and thus, safer nanoparticle alternatives with lower potential health hazards are certainly of interest.

In search for antivirally effective materials with low activity towards human cells or microbiota, we turned our attention to CeO₂-based nanomaterials. A few previous publications demonstrating antiviral activity of ceria nanoparticles against influenza virus H1N1 and herpes simplex virus [30, 31], Sabin-like poliovirus [32] and vesicular stomatitis (VS) virus [31], have been published. Although the mechanism of antiviral activity of CeO₂ nanoparticles has not been thoroughly studied, some information can be derived from studies concerning antibacterial or cellular effects of CeO₂, where the biological activity of CeO₂ has been related to the specific defects in its crystal structure [31]. Such defects have been shown to lead to ox-redox transition between surface Ce(III) and Ce(IV) states resulting in filling or formation of oxygen vacancies [33], and ultimately to the release of reactive oxygen species (ROS) on nanoparticles surface, analogous to TiO₂ [34]. Interestingly, the formation of ROS on the surface of CeO₂ nanoparticles has been linked to the acidity of the surrounding environment. In acidic conditions, the redox transition between Ce(III) and Ce(IV) has been shown to induce the rise in ROS level and thus, toxic effects against bacteria [35] as well as cancer cells [32]. In *in vitro* tests with normal mammalian cells and less acidic pH, no significant cytotoxicity of CeO₂ nanoparticles has been observed but instead, a protective, survival and growth promoting effect has been demonstrated [36, 37]. It has been proposed that these protective effects have been due to entrapment of free radicals by CeO₂ and thus, reduction of ROS-induced oxidative stress [38, 39]. This combination of potential antiviral activity and low general toxicity suggests that CeO₂ materials may be potent antivirals with low side effects.

The aim of this work was to reveal the antiviral activity of CeO₂ ultrasmall nanoparticles towards a selection of enveloped and non-enveloped mammalian viruses and selected bacteriophages. In parallel to CeO₂ nanoparticles we tested the antiviral activity of nanoparticles of silver that are generally considered

to exhibit a significant antiviral activity, and silica (SiO₂) presumably biologically inert [40]. To reveal the cause of antiviral activity of CeO₂ nanoparticles, we studied the potential surface protein inactivation by those nanoparticles using SARS-CoV-2 as an example. Finally, the antiviral potency of CeO₂ and other selected nanoparticles was compared with their bactericidal activity towards Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* and cytotoxic effects.

2. Results And Discussion

2.1. Characterization of nanoparticles

CeO₂ nanomaterials were synthesized with two different states of surface, those with nearly “bare” surface carrying large positive charge (nano-CeO₂(+)) and nanoparticles stabilized by citric ions and, thereby presenting negatively charged surface (nano-CeO₂(-)). Nano-CeO₂(+) could be synthesized at 6500 mg Ce/l (45 mM) and nano-CeO₂(-) particles could be synthesized at maximal concentration of 8200 mg Ce/l (60 mM). Hereafter, in described experiments, mg/l concentration for investigated compounds always refers to milligrams per liter of the element, i.e. Ce, Ag or Si.

As synthesized, nano-CeO₂(+) and nano-CeO₂(-) formed stable aqueous colloids due to their large (+ 41 mV or -53 mV, respectively) ζ-potential (Table 1). According to high resolution transmission electron microscopy (HRTEM) (representative TEM images of CeO₂ nanoparticles are shown on Fig. 1), the particle size of both, nano-CeO₂(+) and nano-CeO₂(-) was close to 3 nm (Figure S1). Dynamic light scattering-measured hydrodynamic size of nano-CeO₂ was relatively close to this (Table 1) and only nano-CeO₂(+) showed some aggregation. Hydrodynamic size of nano-CeO₂ samples was also measured at concentrations used in antiviral testing (Figure S2). Interestingly, apparent increase in particle size and thus, the likelihood for aggregation was observed with decreasing particle concentration for both, nano-CeO₂(+) and nano-CeO₂(-), whereas the latter was more sensitive to dilution-mediated instability. Apparently, the decrease of ionic force upon dilution of colloid lead to the thinning of nanoparticles double electric layer and deteriorated colloidal stability.

Table 1
Key characteristics of nanoparticles used

Nanoparticle	TEM size, nm	Hydrodynamic size ^a , nm	ζ potential, mV
nano-CeO ₂ (+)	3.3 ± 0.4	7.0 ± 3.0	+ 41 ± 2
nano-CeO ₂ (-)	3.2 ± 0.4	4.5 ± 2.0	-53 ± 4
nano-Ag	15.3 ± 3.0	18.5 ± 3.0	-52 ± 5
nano-SiO ₂	16.6 ± 1.9	45 ± 5	-34 ± 3
^a number based hydrodynamic size based on DLS (dynamic light scattering) measurements			

UV-Vis spectra of CeO₂ nanoparticles (Fig. 2A) showed that compared to nano-CeO₂(+) the fundamental absorption edge of nano-CeO₂(-) was approximately 10 nm red shifted. Also, visibly, the colloid of nano-CeO₂(-) showed a deeper yellow color compared to nano-CeO₂(+). This difference in shift of absorption edge may be due to the different Ce(III)/Ce(IV) ratio on the surface of nanoparticles, or originate from oxidized and polymerized citrate ions on the surface of nano-CeO₂(-) particles, as a remnant of the synthesis process. The seeming “drop” of the absorption of nano-CeO₂(-) colloid below 300 nm is a compensation error due to a symmetric rise of absorption of the mother solution (the liquid remaining after centrifugation of nanoparticles) of this colloid caused, most likely, by a high concentration of citrate ions and products of their oxidation and/or complexing with cerium ions. Infrared (IR) spectrum of the synthesized nano-CeO₂ (Fig. 2B) is in agreement with the literature data [41] and exhibits peaks in around 3600 to 3200 cm⁻¹, which correspond to -OH stretching of absorbed water and surface OH-groups. Peaks around 1600 cm⁻¹ and 1400 cm⁻¹ are H-O-H deformational vibrations and C-O stretching vibrations from surface water molecules and carbonate ions, respectively. The peak from 700 to 500 cm⁻¹ is due to Ce-O stretching vibrations. The differences between nano-CeO₂(+) and nano-CeO₂(-) spectra are mainly intensities of peaks around 1600 cm⁻¹ and 1400 cm⁻¹, which in case of nano-CeO₂(-) correspond also to COO-group vibrations of absorbed citrate ions [42]. Also we observed a small blue-shift of the band from 1539 cm⁻¹ nano-CeO₂(+) to 1546 cm⁻¹ for nano-CeO₂(-), which is probably due to summing of H-O-H deformational vibrations with anti-symmetric COO-group stretching band with maximum should be around 1585 cm⁻¹ according to literature data [42]. Together with DLS data, UV-Vis and IR-spectroscopy clearly show, that despite very close mean particle size, shape, and size distribution parameters, nano-CeO₂(+) and nano-CeO₂(-) particles are chemically and electrostatically rather different and, therefore, we can expect from them different effect on biological objects.

Nano-Ag and nano-SiO₂ were synthesized to serve as a positive control, i.e., a potentially antiviral nanoparticle and a negative control, i.e., a presumably biologically inert nanoparticle. Nano-Ag synthesis was carried out through seed-mediated citrate route and the maximum concentration reached was 480 mg/l (corresponds to 4.4 mM). Nano-SiO₂ was synthesized using Stöber technique and the maximum concentration reached for amorphous silica nanoparticles was 3100 mg/l (corresponds to 120 mM). Both, nano-Ag and nano-SiO₂ particles were spherical with average primary size around 15 nm (Table 1). According to hydrodynamic size and DLS analysis, nano-Ag remained in non-aggregated state while nano-SiO₂ exhibited a certain level of aggregation and formation of 2–10 nanoparticle aggregates. The surface ζ-potential of nano-Ag and nano-SiO₂ particles was negative (-52 ± 5 and -34 ± 3 mV, respectively) being comparable to nano-CeO₂(-), thus allowing the comparison of their biological effects. Unfortunately, we were unable to synthesize silica particles with primary size comparable to CeO₂ nanoparticles without the addition of strong surfactants that would have their own pronounced antimicrobial properties.

2.2. Antiviral activity of CeO₂ nanoparticles

For the demonstration of antiviral potency of nano-CeO₂, nano-SiO₂ and nano-Ag, we analyzed the decrease of infective counts (expressed as plaque forming units, PFU) of four mammalian viruses and two bacteriophages after 1 h contact with nanoparticles. Nano-CeO₂ with both positive and negative surface ζ potentials were used to understand the effect of CeO₂ surface in their antiviral activity. To rule out the possibility, that the antiviral activity is only due to the nanometer scale particle size, parallel antiviral activity testing was performed with supposedly biologically inert, low toxicity SiO₂ nanoparticles. Conversely, nano-Ag particles that are widely considered to exhibit antiviral effect, were tested as positive controls in antiviral tests (see Table 2). Despite the very low CeO₂ solubility in water in neutral pH range due to which only $10^{-7} - 10^{-8}$ M released Ce ions concentration is expected in the colloidal solutions of nano-CeO₂ [43], we also tested the antiviral effect of soluble Ce(III) compound Ce(NO₃)₃ as a source of Ce(III) ions. Although theoretically Ce(IV) ions could also form due to dissolution of CeO₂, these are demonstrated to be very unstable in a hydrated state and thus, practically not existing [43].

Our selection of viruses included mammalian and bacterial, both enveloped and non-enveloped viruses (Table 2). In general, enveloped viruses surrounded by a lipid membrane have been considered more sensitive to inactivation by various environmental conditions than non-enveloped viruses possessing only a proteinaceous capsid [44]. Therefore, most of the antiviral testing standards foresee the inclusion of both types of viruses [45, 46]. The enveloped mammalian viruses used in this study included A/WSN/1933 [47], SARS-CoV-2 and TGEV [48], and picornavirus EMCV was used as a model of the non-enveloped mammalian viruses [49]. Enveloped Φ 6 [50] and non-enveloped MS2 [51] were used as respective bacterial virus (bacteriophage) examples. Both of those phages have been suggested as models for antiviral testing [52–55]. In case of all the viruses, exposure to nanoparticles was carried out in sterile water or highly diluted growth medium over 1 h. The numbers of infectious viral particles with and without treatments were expressed as plaque forming units (PFU/ml).

Table 2

Characteristics of viruses and the selection and concentrations of nanoparticles used in antiviral assays.

Virus	Characteristics	Nanoparticle or compound	Purpose	Concentration range tested
A/WSN/1933	Enveloped virus, family <i>Orthomyxoviridae</i> , seasonally important to cause flu in Northern hemisphere	nano-CeO ₂ (+)	Proposed antiviral compound	0.002–2000 µg Ce/ml* 0.002–2000 µg Ce/ml*
SARS-CoV-2	Enveloped virus, family <i>Coronaviridae</i> , causes COVID-19	nano-CeO ₂ (-)	Proposed antiviral compound	0.002–2000 µg Ce/ml*
TGEV	Enveloped virus, family <i>Coronaviridae</i> , transmissible gastroenteritis virus of pigs	Ce(NO ₃) ₃	Control for Ce(III) ion effects	
EMCV	Non-enveloped virus, family <i>Picornaviridae</i> causative agent for neurological disorders	nano-Ag	Nanoparticle with previously demonstrated antiviral activity	0.0015-1500 µg Ag/ml*
Φ6	Enveloped bacteriophage of <i>Pseudomonas sp</i>	nano-SiO ₂	Proposed inert nanoparticle	0.0004-400 µg Si/ml*
MS2	Non-enveloped bacteriophage of <i>Escherichia coli</i>			
*All the tested nanoparticles and chemicals were in the range of 0.014–14000µM				

Prior to antiviral activity testing, the effect of all the tested compounds was studied on viral host cells. Most of the tested compounds and concentrations were non-cytotoxic to the host cells of mammalian viruses (Figure S3). Only the highest tested concentrations of nano-Ag, Ce(NO₃)₃ or SiO₂ decreased the viability of some of the host cell lines, which however did not interfere with antiviral assays, where the concentrations of those compounds did not reach the toxic levels. Except for nano-Ag none of the compounds affected the growth of host bacteria of bacteriophages. As nanosilver is well known for its antibacterial effects, its bacterial toxicity that was observed from 14 µM (1.5 mg/l) was not a surprise. To be able to study the effects of nano-Ag towards bacteriophages, its bacterial toxicity was neutralized by the addition of 3-fold molar excess of L-cysteine to the infection reaction immediately prior the plaque assay step.

The results of antiviral assay showed that despite being non-toxic to mammalian cells and bacteria, nano-CeO₂ affected the infectivity of most of the mammalian viruses and bacteriophages (Fig. 3). Significant decrease ($p \leq 0.05$) of viral infectivity due to 1 h nano-CeO₂(+) exposure was observed starting from 20 mg/l in case of SARS-CoV-2, A/WSN/1933 and $\phi 6$, and starting from 200 mg/l in case of TGEV (Table 3). None of the non-enveloped viruses showed significant decrease in infectivity for nano-CeO₂(+) up to 2000 mg/l. Nano-CeO₂(-) affected the infectivity of SARS-CoV-2 and A/WSN/1933 starting from 20 mg/l and the infectivity of TGEV and $\phi 6$ starting from 200 mg/l (Table 3). Following the trend of nano-CeO₂(+), non-enveloped viruses showed the lowest sensitivity towards nano-CeO₂(-). EMCV infectivity was not affected by nano-CeO₂(-) up to 2000 mg/l, and the response of non-enveloped bacteriophage MS2 to nano-CeO₂(-) was non-monotonic so that 20 and 200 mg/l of the compound decreased the infectivity, but no effect was observed at 2000 mg/l. Such a non-monotonic effect on antiviral activity could not be explained by increasing aggregation level of CeO₂ at higher concentration as according to hydrodynamic size measurement, as nano-CeO₂ were non-aggregated at higher concentrations and particle aggregation and instability increased with dilution level of the particles (Figure S2). Non-monotonic response has been shown also for other types of nanomaterials. For example, TiO₂ exhibits non-monotonic UV light-induced toxicity to freshwater organisms [56]. In case of CeO₂ nanoparticles, earlier reports can be found on non-monotonic toxicity towards soybean plants [57], however, its exact causes are still to be clarified.

Although we proposed that due to the chemical and electrostatic differences between the surface of nano-CeO₂(+) and nano-CeO₂(-) those nanoparticles may exhibit different biological effects, our results showed no notable differences between the antiviral properties of those two types of particles (Table 2).

Comparison of our results on antiviral properties of nano-CeO₂ with earlier reports is rather difficult due to the variable nature of the nanoparticles, as well as methods used for antiviral activity assessment. However, certain comparisons may be made. For example, in a 2010 study, 2 log reduction of enveloped *Enterobacter aerogenes* infecting bacteriophage UZ1 PFUs was observed after its 2-h exposure to 50 mg/l CeO₂ whereas 500 mg/l of CeO₂ was sufficient to decrease infectivity by 4 logs [58]. Mohamed et al. [32] showed that 14 nm sized nano-CeO₂ at concentrations less than 50 mg/l removed all the infectious viral particles (PFU) of type 1 Sabin-like poliovirus and the authors even suggested CeO₂ nanoparticles as an alternative to treatment of polio infection. Yet there are also reports showing no effect by CeO₂ nanoparticles on viruses. According to Neal et al. [59] 200 mg/l of CeO₂ nanoparticles did not have any significant effect on infectivity of SARS-CoV-2 OC43 or rhinovirus RV14 after 6 h incubation. However, after doping of CeO₂ with Ag, antiviral activity of CeO₂ nanoparticles was achieved, according to authors, due to the presence of increased proportion of Ce(III) ions on the nanoparticles surface. Overall, these few articles published so far on antiviral efficacy of CeO₂ have demonstrated relatively variable results, which may be attributed to differences in physico-chemical properties of nanoparticles, to different viruses used or antiviral assays applied. However, based on the few earlier studies demonstrating antiviral effects of

nano-CeO₂ [32, 58] and the results of our study, we may suggest that nanoceria has a significant potential in antiviral treatments and that this potential should be studied and developed further.

Comparison of the antiviral efficacy of nano-CeO₂ in comparison with nano-SiO₂ that was used as a negative control, and nano-Ag used as a positive control due to its likely antiviral properties are shown in Fig. 4. Expectedly, the non-enveloped viruses that did not show any sensitivity towards nano-CeO₂ were also not influenced by nano-SiO₂ or nano-Ag (Fig. 4D and F). The inexistent antiviral effect of SiO₂ nanoparticles was expected as silica is considered biologically compatible or generally regarded as safe [60, 61] and of low toxicity. We found no published data that would have indicated an antiviral activity of SiO₂ nanoparticles. Yet silica nanoparticles have been shown to impede antiviral response to norovirus infection, reducing the viability of macrophages [62].

While the low antiviral activity of SiO₂ was anticipated, our results indicating no significant virucidal activity of nano-Ag up to 150 mg/l (1.4 mM), except for bacteriophage ϕ 6 (Table 2) was surprising. Most unexpected was the result showing the absence of the antiviral activity towards coronaviruses (SARS-CoV-2 and TGEV) and influenza virus A/WSN/1933. Earlier studies have suggested that nanosilver may affect viruses in a variety of ways; through interactions with viral surface and subsequently interfering the viral attachment to its targets, inhibition of viral penetration into host cells or even by interacting with viral genome [27], and that silver nanoparticles are affecting viruses already starting from tens of mg/l. However, interestingly, a closer look to Ag nanoparticles antiviral results revealed that in most of the studies only a relatively small, less than 1 log (< 90%) decrease in infectivity has been observed. Yadavalli et al [9] have shown inhibition of 50% HIV by Ag nanoparticles between 25 and 5000 mg/l, Rogers et al [25] have demonstrated 60–80% decrease in monkeypox virus plaque formation activity in the presence of 20-2000 mg/l of Ag nanoparticles. Up to 90% inhibition of human parainfluenza virus in the presence of 0.1-9 mg/l Ag nanoparticles has been demonstrated by Gaikwad et al. [63]. Slightly higher, up to 2 log decrease of influenza virus titer was observed in response to 50–70 mg/l Ag nanoparticles treatment [64] and Castro-Mayorga et al [21] demonstrated 4 log decrease of median tissue culture infectious dose of feline calicivirus after exposure to 10 mg of nano-Ag/l. Considering that the decrease of viral titers ≥ 2 log can be regarded as the lowest biologically meaningful activity in antimicrobial applications and at least 4 log reduction within up to 1 h is required for biocidal products in suspension tests in the framework of European legislation [65], there is only little proof that Ag nanoparticles would be effective antivirals from the point of view of these requirements. Therefore, our conclusion on low antiviral activity of nano-Ag in terms of log PFU decrease during 1 h exposure (Table 2) is at broad level in line with the previous reports. However, in general view this conclusion is rather surprising as it does not prove the antiviral efficacy of nanosilver contrary to the common belief.

Table 3. Log decrease of infectious viral titer or viable bacterial count at different concentrations of nanoparticles and Ce(NO₃)₃ (numerical data from Figures 3 and 4). Concentrations causing at least 1 log (90%), 2 logs (99%), 3 logs (99.9%) and 4 logs (99.99%) decrease are indicated with light to dark green color gradient. A minimum of 2 log reduction (99%) in viable bacterial count or infectious viral titer is

generally considered as lowest biologically meaningful activity in antimicrobial applications while at least 4 log reduction within up to 1 h is required from biocidal products in suspension tests in the framework of European legislation [65]. * p<0,05, ** p<0,01, *** p<0,001 and **** p<0,0001.

Concentration mg/L (μM)	Enveloped				Non-enveloped		Bacteria	
	Viruses		Bacterio- phages		Viruses	Bacterio- phages	Gram- negative	Gram- positive
	SARS- CoV-2	TGEV	A/WSN/1 933	Φ6	EMCV	MS2	<i>E. coli</i>	<i>S. aureus</i>
nano-CeO₂(+)								
2 (14)	0.0	-0.1	0.1	0.2	-0.2	-0.2	0.32	0.14
20 (142)	1.1 *	-0.2	1.5****	4.4****	-0.3	0.1	0.94*	0.07
200 (1427)	1.4 **	1.1***	1.6****	6.4****	-0.2	0.4	1.03*	0.06
2000 (14 270)	6.7 ****	3.2****	4.3****	6.4****	-0.3	0.4	0.43	0.11
nano-CeO₂(-)								
2 (14)	0.1	0.0	0.4	-0.2	-0.1	0.4	1.52****	0.10
20 (142)	1.4 **	0.2	2.0****	0.0	-0.1	1.6***	1.68****	0.13
200 (1427)	2.3 ****	2.2****	2.4****	2.9****	-0.1	1.4**	1.48***	0.02
2000 (14 270)	3.3 ****	2.3****	4.3****	2.8****	0.2	0.1	0.18	-0.03
Ce(NO₃)₃								
2 (14)	0.0	0.1	0.4	0.1	-0.4	-0.2	1.68****	0.05
20 (142)	0.9	0.2	3.3****	3.3****	-0.3	0.0	2.83****	0.15
200 (1427)	1.9 ****	0.9	4.7****	6.0****	-0.1	0.4	3.52****	0.25
2000 (14 270)	na	1.2*	4.7****	6.4****	-0.1	0.8	6.76****	1.02**
nano-Ag								
1.5 (14)	0.0	0.0	0.3	5.3****	-0.1	-0.2	6.70****	0.40
15 (140)	-0.2	0.0	0.2	6.3****	0.0	-0.2	6.70****	7.29****
150 (1400)	0.0	0.3	0.5	6.3****	-0.2	-0.2	6.70****	7.29****
1500 (14 000)	na	na	na	na	Na	na	na	Na
nano-SiO₂								
0.4 (14)	0.0	0.0	0.0	-0.5	0.0	-0.1	-0.13	0.01
4 (140)	0.0	-0.1	0.1	-1.1	0.0	-0.1	-0.29	0.10
40 (1400)	0.1	0.1	0.4	-1.1	0.1	-0.2	-0.44	0.24
400 (14 000)	0.2	0.5	0.9	-1.1	0.1	-0.1	-0.38	0.04

2.3 Disruption of SARS-CoV-2 binding to ACE2 receptor by nano-CeO₂

To date there is no single mechanism of action proposed as the basis of antiviral activity of CeO₂ nanoparticles. One theoretical mechanism of action of CeO₂ nanoparticles could be via released Ce ions [66]. However, even though the release of Ce(III) ions may take place, the equilibrium concentration of released ions has been shown extremely low [43, 67]. In order to test the possibility that nano-CeO₂ exhibited antiviral activity due to released Ce(III) ions, we analyzed the viral response to a soluble Ce salt, Ce(NO₃)₃. The antiviral activity of Ce(NO₃)₃ (Fig. 4) was seen only at concentrations of Ce(III) ions several orders higher than achievable due to dissolution of CeO₂ nanoparticles, thus, excluding the possibility of noticeable influence of Ce(III) ions on the antiviral activity of CeO₂.

As a different mechanism of inactivating viruses, binding of CeO₂ nanoparticles on genetic material has been reported. Link et al. [68] showed high binding capacity of nanoparticles of CeO₂ for nucleic acids in adeno-associated virus, adenovirus, human immunodeficiency virus, and murine leukemia virus [68]. In a similar line, Neal et al [59] proposed that Ag-doped CeO₂ nanoparticles may act via physical interactions between the particles and the membrane of enveloped viruses, leading to disruption of the lipid bilayer of enveloped viruses. In case of non-enveloped viruses, the particles were proposed to interact with virion proteins.

In order to control the potential of nano-CeO₂ binding to virions and by that, interfering with their natural ligand binding, we performed an ELISA assay to measure the *in vitro* binding activity of nano-CeO₂ exposed SARS-CoV-2 to its target, the human recombinant ACE2 receptor (Fig. 5). Interestingly, exposure of SARS-CoV-2 to nano-CeO₂(-) and nano-CeO₂(+) affected the virus somewhat differently. While exposure of SARS-CoV-2 to ≥ 200 mg/l of nano-CeO₂(-) inhibited the binding of the virus to ACE2, exposure of SARS-CoV-2 to nano-CeO₂(+) had no observable effect on the ability of the virus to bind recombinant hACE2. However, as the antiviral profiles of both CeO₂ nanoparticles were relatively similar, these results do not allow us to claim that surface binding and blocking of ligand binding are the leading mechanism of antiviral action of nano-CeO₂ evidenced by us in antiviral assays. Therefore, the mode of action driving the antiviral effect of CeO₂ nanoparticles is to be elucidated in future studies.

2.4. Bactericidal effect of CeO₂ nanoparticles

In parallel to antiviral properties, antibacterial activity towards the Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* was analyzed. According to our results, neither nano-CeO₂(+) nor nano-CeO₂(-) up to 2000 mg/l decreased the viable counts (expressed as colony-forming units, CFU) of *E. coli* or *S. aureus* by more than 2 logs (99%) (Fig. 6, Table 3), indicating that the antiviral effect of CeO₂ nanoparticles was more pronounced than their bactericidal efficacy. Comparison of smaller changes than 2 log decrease in CFU levels in *E. coli* and *S. aureus* showed that Gram-negative *E. coli* was affected by nano-CeO₂ to a higher extent than Gram-positive *S. aureus* (Table 3). While nano-CeO₂(-) concentrations of 2, 20 and 200 mg/l decreased the number of *E. coli* CFUs by 1.48–1.68 logs, no decrease in viable counts of *S. aureus* in response to nano-CeO₂ was observed. Interestingly, the response of *E. coli* was non-monotonous as also in case of non-enveloped bacteriophage MS2. The higher sensitivity of Gram-

negative bacteria compared with Gram-positive bacteria against nano-CeO₂ particles has been shown also in earlier studies and has been related to redox activity of nanoceria and the ability of a thicker peptidoglycan layer to alleviate this effect [69]. In the literature, variable results can be found on antibacterial effects of CeO₂ nanoparticles, both due to the variety of nanoparticles used as well as due to the different antibacterial assays applied. The minimal inhibitory concentrations (MIC) of nano-CeO₂ towards a series of Gram-positive and -negative bacteria has been shown to vary between 20 and 140 mg/l [70, 71]. Another study where nanoparticles of CeO₂ were prepared using surfactant Tween-80, demonstrated MIC of 150 mg/l whereas without the surfactant the MIC value was 3000 mg/l [72]. In another study, complete inactivation of *E. coli* was achieved in the presence of 1000 mg/l of CeO₂ nanoparticles [73]. Thus, our result showing no significant antibacterial activity of CeO₂ towards Gram-positive *S. aureus* (Table 2) and relatively modest antibacterial effect towards Gram-negative *E. coli* up to 2000 mg/l, coincided with some of the results published earlier but differed from others, respectively.

Differently from nano-CeO₂ that showed significant or relatively modest antibacterial effect, nano-Ag particles demonstrated significant antibacterial effect already at 1.5 mg/l. Expectedly Gram-negative bacteria were more sensitive towards nano-Ag and at 1.5 mg/l, already more than 4 logs decrease in *E. coli* viable counts was observed. Viability of Gram-positive *S. aureus* decreased by > 4 log from 15 mg nano-Ag/l (Fig. 6, Table 3). These results are in agreement with earlier studies that have shown the efficacy of Ag nanoparticles starting from low mg/l range [28]. Indeed, silver nanoparticles that are shown to act via silver ion release and ROS formation [74] and the following interaction between Ag ions and thiol groups of proteins as well as permeabilization of bacterial membrane, have been generally regarded as the nanoparticles with highest antibacterial activity. In January 2022, more than 18,000 articles were registered in ISI WoS on “nano* AND silver* AND antibacter*”.

Nanoparticles of SiO₂, generally regarded as harmless, did not show any bacterial toxicity in our experiments. Inexistent antibacterial activity of nano-SiO₂ alone has been shown also in other studies, however, most of the papers on SiO₂ used silica nanoparticles as carriers of more biologically active metal ions or other nanoparticles and thus, are not an adequate comparison for our purpose.

3. Conclusion

This study focused on antiviral effects of CeO₂ nanoparticles, which have been shown earlier to exhibit controversial biological effects, including antibacterial and antiviral activity presumably due to their redox activity, and reactive radical scavenging activity in mammalian cells. Our antiviral assays with nano-CeO₂ exhibiting two different surface charge and surface functionality showed that both CeO₂ nanoparticles exhibited antiviral properties and those did not depend on the surface charge or surface functional groups suggesting that the intrinsic properties of the nanoparticles were responsible for the antiviral effect. Using four enveloped viruses we were able to demonstrate more than 2 logs (99%) decrease in

viral infectivity, in the presence of 20 or 200 mg/l of nano-CeO₂ and more than 4 logs (99.99%) decrease in infectious viral counts at 200–2000 mg/l. At the same time, the maximum decrease in viable counts of *Escherichia coli* bacteria was only 97% and the antibacterial effect was non-monotonous Gram-positive bacterium *Staphylococcus aureus* was not affected by nano-CeO₂ at any of the tested concentrations. Importantly, no cytotoxic effect of nano-CeO₂ was observed. On the other hand, Ag nanoparticles that have been claimed antiviral by several previous reports, did not show any significant antiviral activity in our study, except for a non-enveloped bacteriophage ϕ 6. However, nano-Ag proved antibacterial and reduced the viable counts of bacteria by more than 99.99% already at low mg/l concentrations. At higher concentrations, nano-Ag exhibited also cytotoxic effects. Thus, based on our results we can conclude that Ag nanoparticles exhibit a relatively unspecific biological effect while nano-CeO₂ show a relatively specific antiviral activity. We also demonstrated that the antiviral activity detected in this study was likely not due to the binding of nano-CeO₂ onto the viral surface and resulting effect on the binding of viral particles to their cellular ligands, nor due to Ce(III) ions released from the surface of CeO₂ nanoparticles. Therefore, to further specify the mechanism of antiviral action of nano-CeO₂ particles, a more detailed research is needed to elucidate the specific targets of nanocerium in different viruses.

4. Materials And Methods

All chemicals used in the study were of analytical grade. Components of growth media are described in more detail below.

4.1. Synthesis of CeO₂, Ag and SiO₂ nanoparticles

The target materials which antiviral potential was assessed in this study were nanoparticles of ceria that were synthesized using two methods resulting in nano-CeO₂ with positive and negative surface zeta potential. The antiviral effects of nano-CeO₂ were compared with nanoparticles of silica (nano-SiO₂) that were considered biologically inert (negative control) and silver nanoparticles (nano-Ag) that were considered potentially antiviral (positive control). The following chemicals were used for the synthesis of nanoparticles: cerium (III) nitrate hexahydrate (99.0%, Sigma-Aldrich), diammonium cerium (IV) nitrate (98%, Fluka), hexamethylenetetramine (HMTA, analytical grade, Sigma-Aldrich), citric acid (99%, Fluka), sodium citrate dihydrate (98%, Sigma-Aldrich), tetraethyl orthosilicate (TEOS, 98%, Fluka), 0.1 M water solution of AgNO₃ (Fluka, analytical grade), sodium tetrahydridoborate (Sigma Aldrich, reagent grade), ethyl alcohol (analytical grade, Sigma-Aldrich), polyethylene glycol (PEG, M_w 1500, Reagent grade, Sigma Aldrich), polyvinylpyrrolidone (PVP, M_w 10000, Reagent grade, Sigma Aldrich), 30% aqueous ammonia solution (Reagent grade, Sigma Aldrich). All solutions were made with MQ deionized water.

For the synthesis of nano-CeO₂(+) nanoparticles the modified technique from [75] was used. Diammonium cerium (IV) nitrate was hydrolyzed at high temperature in the presence of HMTA. For that, 0.189 g of (NH₄)₂Ce(NO₃)₆ and 0.053 g of HMTA were dissolved in 50 ml of water and loaded into an

autoclave vessel (100 ml). The vessel was sealed and heated to 180°C for 30 minutes in the microwave-hydrothermal device (Berghof Speedwave 4, 2.45 GHz, 1000 W). After thermal treatment, the vessel was cooled down to room temperature in a water bath. The product was centrifuged for 10 minutes, the sediment was washed with deionized water and redispersed by ultrasonication. These steps were repeated at least 3 times and the final product was redispersed in 5 ml of MQ water by ultrasonication until opalescent pale-yellow colloid was obtained. As a result, nanoparticles with an almost “bare” surface carrying positive charge were obtained.

For the synthesis of nano-CeO₂(-) nanoparticles we used a method from [76]. Cerium(III) nitrate was hydrolyzed at room temperature in the presence of ammonia with simultaneous oxidation by oxygen from the air. For that, 0.045 g of citric acid was dissolved in 25 ml of prepared in advance 0.05 M aqueous solution of cerium (III) nitrate. The solution was rapidly added into 100 ml of 3 M solution of ammonia and left to stir vigorously for 2 hours during which the color of solution changed from colorless to yellow-orange. After that the colloid solution was centrifuged, washed and redispersed by ultrasonication. These steps were repeated at least 3 times and the final product was redispersed in 15 ml of MQ water by ultrasonication until transparent dark yellow colloid was obtained. This method allowed the production of ceria nanoparticles stabilized by citrate-ions and therefore carrying a large negative charge.

For synthesis of nano-Ag a two-step seed-mediated technique was used adopted from [77] and modified to avoid toxic reagents. For preparation of seeds 20 ml of 2.5 mM solution of sodium citrate were mixed with 1 ml of 5g/l aqueous PVP (MW 10000) solution and 1.2 ml of freshly prepared 10 mM solution of NaBH₄. To this mixture 20 ml of 0.5 mM solution of silver nitrate was added at the rate of 2 ml/min under constant stirring. The Ag seeds were left to form for 15 min under stirring at room temperature. In the second stage 27 ml of prepared seed solution was added to the solution of 0.5884 g of sodium citrate in 470 ml of MQ water. This mixture was heated up to 85°C under stirring and when this temperature was reached, 0.625 ml of 0.1 M AgNO₃ solution was added to the solution, which was afterwards left under stirring for 15 min. In order to purify and concentrate the obtained solution, ultracentrifugation was performed (Beckman Coulter, Optima XE-90 Ultracentrifuge device, 28000 rpm/141000 g, 2.5 hours). This allowed us to obtain a solution with 0.13 g/ml concentration. From this stock solution, less concentrated samples were prepared by dilution with MQ water.

Synthesis of nano-SiO₂ was carried out using a modified Stöber technique [78]. For that, 18 ml of MQ water was mixed with 100 ml of ethanol. In this water-alcohol mixture 1.5 g of PEG, 6.2 ml of TEOS and 3 ml of NH₄OH solution were dissolved. The resulting reaction mixture was left at room temperature under vigorous stirring for 24 hours. After that the synthesized SiO₂ nanoparticles were centrifuged, washed with deionized water and redispersed by ultrasonication. These steps were repeated at least 3 times and the final product redispersed in 5 ml of MQ water by ultrasonication until opalescent colorless colloid was obtained.

4.2. Characterization of CeO₂, SiO₂ and Ag nanoparticles

Particle size and morphology were studied by means of transmission electronic microscopy (FEI Tecnai F20 TEM). For TEM analysis 85 mg/l particle suspension was deposited onto a 400 mesh holey carbon coated copper grid (Agar scientific AGS147-4). DLS and zeta potential measurements were done with Malvern Zetasizer Nano ZSP. The measurements of nano-SiO₂ and nano-Ag were carried out using corresponding pre-set parameters of the device; nano-CeO₂ measurements were carried out using pre-set parameters for TiO₂ nanoparticles as the closest among available. All measurements were performed in water.

UV-Vis spectra were obtained using the Agilent Cary 5000 UV-Vis-NIR device. The measurement range for UV-Vis was from 800 nm to 250 nm. FTIR measurements were performed with the Bruker Vertex 70 FTIR spectrometer. The ATR configuration was used with diamond crystal. Detectors used were a mercury cadmium telluride and MCT detector. Data was obtained from the range of 4000 cm⁻¹ to 400 cm⁻¹.

4.3. Toxicity testing of nanoparticles on viral host organisms

Prior to antiviral activity testing, the effects of nanoparticles and other test chemicals were assessed on host organisms of those viruses - cell lines in case of mammalian viruses and bacteria in case of bacteriophages.

Cytotoxicity testing with mammalian cell lines. Virus host cell lines were tested (Table 4). Cells were seeded onto 96-well plates and grown in specified medium (Table 1) to reach 70% confluency after which growth medium was removed and 100 µl of the tested compound corresponding to that later tested for antiviral effects was added. The cells were incubated for 1 h at 37°C, 5% CO₂, humidified atmosphere after which the medium with the test compounds was removed, fresh growth medium was added and cells were further incubated for 48 h. Cell viability was measured using Cell Proliferation Reagent (WST-1) (Roche) to which 10 µL/well WST-1 reagent was added, the plate was incubated at 37°C, 5% CO₂ for 3 h and absorbance at 450 nm was read. For calculation, the value of a blank well (no cells present) was subtracted from each measurement and the value of wells without a test compound was considered as 100% viability. Three replicates were tested for each compound and concentration. The concentrations of test substances that resulted in cellular toxicity were not tested for their antiviral effects as interferences from cellular toxicity could not be avoided.

Toxicity to host bacteria. Host bacteria for the two bacteriophages (Table 4) were exposed to the test substances in plaque assay format. Bacterial lawns were created by mixing overnight culture of *Pseudomonas* or exponentially grown culture of *Escherichia coli* with TSB soft agar (Table 4) and poured to Petri dishes as described under 4. *Bacteriophages*. 10 µl drops of the specified concentration of test substances (see the tested concentration ranges in Table 2) were pipetted onto the agarized surface. After overnight incubation, bacterial lawn was observed for inhibition zones at the drops of chemicals. None of the nanoparticles or chemicals except nano-Ag resulted in inhibition zones. To mitigate nano-Ag toxicity in antiviral assay with bacteriophages these nanoparticles were tested in combination with

threefold molar excess of L-cysteine. Thus, in case of 14 μM nano-Ag equal volume of 42 μM L-cysteine was added, in case of 140 μM nano-Ag equal volume of 420 μM L-cysteine was added and in case of 1400 μM equal volume of nano-Ag 4200 μM L-cysteine was added. L-cysteine concentrations till 4200 μM did not decrease the titer of neither of the bacteriophage.

Table 4
Viruses, their host organisms and maintenance conditions

Virus	Titer in exposure, PFU/ml	Host organism	Maintenance/growth conditions
Enveloped viruses			
Mammalian viruses			
SARS-CoV-2	5*10 ⁵	Vero E6 cells (Growth medium: DMEM, 10% heat-inactivated FBS, 100 U/ml penicillin and 100 µg/ml streptomycin; grown at 37°C and 5% CO ₂)	Virus growth medium: DMEM, 0,2% BSA, 100 U/ml penicillin, 100 µg/ml streptomycin
TGEV	8,35*10 ⁶	ST cells (Growth medium: DMEM, 10% heat-inactivated FBS, 100 U/ml penicillin and 100 µg/ml streptomycin, 37°C 5% CO ₂)	Virus growth medium: DMEM, 0,2% BSA, 100 U/ml penicillin and 100 µg/ml streptomycin
A/WSN/1933	6,65*10 ⁴	MDCK-2 cells (Growth medium: DMEM, 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin and 100 µg/ml streptomycin, 37°C 5% CO ₂)	Virus growth medium: DMEM, 0,2% BSA, 100 U/ml penicillin and 100 µg/ml streptomycin, 1 µg/ml TPCK-treated trypsin
Bacteriophages			
ϕ6	1*10 ⁷	<i>Pseudomonas sp</i> (Growth medium Tryptone Soy Broth TSB: 17 g/l casein peptone, 3 g/l soy peptone, 2.5 g/l glycose, 5 g/l NaCl, 2.5 g/l K ₂ HPO ₄ ; 25°C; for solid medium 15 g/l agar was added)	Phage maintenance medium: SM buffer (0.1 M NaCl, 8 mM MgSO ₄ , 50 mM Tris-HCl; pH = 7.5 Semisolid TSB top agar used for infection: 7 g/l agar added to TSB
Non-enveloped viruses			
Mammalian viruses			
EMCV	2,5*10 ⁷	BHK-21 cells (Growth medium: GMEM, 10% heat-inactivated FBS, 2% TPB, 2% 1 M Hepes (pH 7,2), 100 U/ml penicillin, 100 µg/ml streptomycin, 37°C 5% CO ₂)	Virus growth medium: GMEM, 0,2% BSA, 2% 1 M Hepes (pH 7,2), 100 U/ml penicillin, 100 µg/ml streptomycin
Bacteriophages			

Virus	Titer in exposure, PFU/ml	Host organism	Maintenance/growth conditions
MS2	1×10^7	<i>Escherichia coli</i> (Growth medium NZCYM broth: 10 g/l casein hydrolysate, 5 g/l NaCl, 1 g/l casamino acids, 5 g/l yeast extract, 2 g/l $\text{MgSO}_4 \times 7\text{H}_2\text{O}$, 2 g/l maltose 37°C; for solid medium 15 g/l agar was added)	Phage maintenance medium: SM buffer (see above) Semisolid TSB top agar used for infection: 7 g/l agar added to NZCYM

4.4. Testing of antiviral activity

The six viruses and bacteriophages used in the tests and media used for their infection and propagation are described in Table 4 and below. Nanoparticles and chemicals used for antiviral testing are shown in Table 2. In general, aqueous suspensions of chemicals at specified concentrations were mixed with an equal amount of bacteriophages in water or with mammalian viruses in 1/10 water-diluted cell culture medium. Samples were incubated for 1 h at room temperature after which these were diluted in 10-fold series using SM buffer (bacteriophages) or virus growth medium (mammalian viruses).

Mammalian viruses. Viruses (Tables 2 and 4) were obtained from the following sources: SARS-CoV-2 was a local Estonian isolate obtained from Estonian Health Board; A/WSN/1933 was from SinoBiological; TGEV was obtained from L. Enjuanes at Department of Molecular and Cell Biology, National Center of Biotechnology (CNB-CSIC), Madrid, Spain; EMCV was obtained from A. Kohl MRC-University of Glasgow Centre for Virus Research, Glasgow, Scotland, United Kingdom. The cell lines for virus propagation (Table 4) were from the cell culture library of Tartu University Institute of Technology, except for ST cells obtained from L. Enjuanes, Madrid, Spain. For antiviral activity testing, the viruses with initial titers in virus growth media (Table 4) were diluted 1:10 with sterile water, mixed with the test compound or chemical and incubated as above. After incubation, the virus mixtures were diluted with virus growth medium by 10 times and 150 µl of the resulting sample was used to infect 100% confluent host cells grown on 12- or 96-well plates and washed with phosphate buffered saline (PBS). In SARS-CoV-2 experiments, 25 µl of the diluted virus sample was used to infect Vero E6 cells. 150 µl or 25 µl of virus growth medium was added to cells in negative control. Infection was carried out at 37°C, 5% CO_2 , humidified atmosphere for 1 h with gentle rocking every 10 min. After 1 h, the infection medium was removed and 3:2 mix of virus growth medium: 2% carboxymethyl cellulose (CMC) was added (in case of A/WSN/1933 the mix was supplemented with 1 µg/ml TPCK-trypsin). Cells were grown in respective growth media for 96 h (as an exception 48h in case of EMCV and Vero E6) at 37°C, 5% CO_2 , humidified atmosphere. For plaque counting in ST, MDCK-2 and BHK-21 cells, the virus growth medium was removed, and plates were dyed using crystal violet. Plaques were identified as clear plaques within the cell layer. In Vero E6 cells, mini-plaque method was used for which plates were fixed with ice-cold 80% acetone in 1x PBS at -20°C for 1 hour. Acetone was then removed by pipetting and the plates were dried for at least 3 hours. Dried plates were treated with a blocking buffer (Thermo Scientific, Ref. 37587)

diluted in PBS/0.05% Tween20 (PBS-T) for 60 min at 37°C. Next, the plates were probed with rabbit monoclonal anti-SARS-CoV-2 nucleocapsid antibody 82C3 (Icosagen AS, ref. nr. R1-166-100) diluted in PBS-T for 1 hour at 37°C. The plates were washed 6x 5 minutes with PBS-T and treated with secondary goat anti-rabbit IRDye800CW antibody (LI-COR Biosciences, ref. nr. 926-32211) diluted in blocking buffer for 60 min at 37°C. Plates were washed with PBS-T 6x 5 minutes, dried and scanned using LI-COR Biosciences Odyssey Infrared Imaging System and application software, to identify fluorescent focuses (mini-plaques). Minimum 3 plaques and maximum of 30 plaques per well were counted and PFU value was calculated according to the dilution and the volume of the inoculum. Each concentration of nanoparticles or salt was tested in three replicates and at least three independent experiments were performed.

Antiviral activity was calculated as a difference between log-transformed plaque forming unit (PFU) counts in negative control and the test sample. 2-log decrease in PFU counts during 1 hour was considered as the lowest biologically meaningful threshold in potential applications.

Bacteriophages. The enveloped bacteriophage $\phi 6$ (DSM21518) and non-enveloped MS2 (DSM21428) and their host bacteria, *Pseudomonas sp* (DSM21428) and *Escherichia coli* (DSM5695) were from German Collection of Microorganisms and Cell Cultures (DSMZ). Bacteriophages were propagated in their host bacteria and purified in titers shown in Table 4. Bacteriophages in water were exposed to compounds and chemicals as above for 1 h after which SM buffer was used to dilute phages for plaque assay. For infection, 10 μ L of bacteriophage samples were dropped as ~ 0.5 cm wide circles onto bacterial plates prepared from overnight grown culture (*Pseudomonas sp*) or 2 h grown exponential culture prepared from overnight culture (*Escherichia coli*). 125 μ L of bacteria were mixed with 5 ml TSB medium in 0.7% agar (TSB semisolid top agar) at 40°C and poured onto LB agar plates as a thin layer. 10 μ L of phage that was incubated in water without compounds as dropped onto bacterial lawns for negative control. Plaques (areas with no visible growth of the host bacterium) within the circles were counted after overnight incubation to obtain the plaque forming units PFU/ml. Minimum of 3 plaques were counted per circle. Each concentration of nanoparticles or chemicals was tested with two biological replicates and at least three independent experiments were performed.

4.5. Bactericidal assays

In antibacterial tests *Escherichia coli* DSM1576 (ATCC8739) and *Staphylococcus aureus* (ATCC6538) originating from American Type Culture Collection were used. Nanoparticles and $\text{Ce}(\text{NO}_3)_3$ at specified concentrations were mixed with an equal volume of bacterial suspensions in water. Bacterial suspensions with 1×10^7 CFU/ml were prepared by cultivating the bacteria in their growth media (Lysogeny broth (LB): 10 g/l tryptone, 5 g/l yeast extract, 10 g/l NaCl) and subsequent washing with water (5 min centrifugation, resuspension in water and following centrifugation). Bacteria with specified concentrations of nanoparticles and chemicals were incubated for 1 h at room temperature after which the samples were diluted in 10-fold series using PBS and drop-plated for viable cell counting (colonies were counted after overnight growth) as in [79]. Minimum of 3 colonies were counted per drop. Each

condition was tested with two biological replicates and at least three experiments were performed. Antibacterial activity was calculated as a difference between log-transformed colony forming units (CFU) in negative control and the test sample.

4.6. ELISA assay to assess receptor binding of SARS-CoV-2 in the presence of nano-CeO₂

Binding of nano-CeO₂ particles to virus surface proteins was analyzed using SARS-CoV-2 and its cellular receptor human recombinant ACE2-hFc. Maxisorp (Nunc) ELISA plates were covered with ACE2-hFc (product P-308-100, Icosagen, Estonia) in PBS at 1 µg/well, and incubated at + 4°C overnight. The plate was washed with PBS-T (phosphate-buffered saline with addition of 0.05% Tween20) 5x 5 min, blocked with 3% BSA/PBS at + 4°C overnight and then washed with PBS-T. 10 µl of nano-CeO₂(+) and (-) at varying concentrations was mixed with 10 µl (5x10⁴ PFU, i.e., 5x10⁵ PFU/ml) of recombinant SARS-CoV-2 (rescued from infectious clone constructed at the Tartu University Institute of Technology; contains mutations in SARS-CoV-2 S-protein from South African isolate). Mixtures were incubated at room temperature for 1 h. 4 µl of virus-nanoparticle mixture was diluted 25 times and transferred to the plate coated with recombinant hACE2 receptor. The plate was incubated at 37°C overnight, fixed with ice-cold 80% acetone/PBS at -20 C for 1 hour. Acetone was removed and the plate dried for 1 h. After that blocking with 3% BSA/PBS was carried out at + 37°C for 1 h. Primary antibody, rabbit monoclonal antibody against SARS-CoV2 RBD 72A5 B12 (R1-172-100 Icosagen, Estonia) was diluted in 3% BSA/PBS-T 1:5000 and placed onto the ELISA plate at + 4°C overnight. Then, the plate was washed with PBS-T 5x 5 min and incubated with secondary antibody, anti-rabbit-HRP (horseradish peroxidase conjugate, LabAS, Tartu, Estonia) diluted 1:10000 in 3% BSA/PBS-T at + 37°C for 1 hour. The plate was then washed with PBS-T 5x 5 min and 50 µl/well of 3,3', 5,5'-Tetramethylbenzidine substrate (Bio-Rad) was added, the wells were incubated at room temperature for 15 min. The reaction was stopped by adding 50 µl/well of 0.5 M H₂SO₄. Absorbance at 450 nm was quantified using a microplate reader. Wells non-treated with hACE2 were used as negative control.

4.7. Statistical analysis

Log-transformed data was used for the analysis of PFU or CFU counts. Two-way ANOVA analysis followed by Dunnett's *post-hoc* test to detect significant differences from control and corrected for multiple comparisons at 0.05 significance level was executed in GraphPad Prism 9.3.0. Only statistically significant differences are marked in Table 3.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The data are available upon request from the authors. Or doi?

Competing interests

There are no competing interests

Funding

The authors gratefully acknowledge the financial support by the Estonian Research Council Grants (COVSG2, PRG629, PRG1496), Estonian Centre of Excellence in Research project “Advanced materials and high-technology devices for sustainable energetics, sensorics and nanoelectronics” TK141 (2014–2020.4.01.15–0011) and University of Tartu Development Fund (PLTFYARENG53). The research was partly conducted using the NAMUR+ core facility funded by projects „Center of nanomaterials technologies and research” (2014-2020.4.01.16-0123) and TT13.

Authors' contributions

AI was responsible for securing the funding, designing the study and writing the manuscript, AN was responsible for nanoparticles synthesis and characterization, DLS analysis, writing and editing the manuscript, KR was responsible for most of the antiviral tests and cytotoxicity assays as well as editing the manuscript, EZ was responsible for antiviral testing with SARS-CoV-2 and ELISA assays as well as for writing and editing the manuscript, AV was responsible for nanoparticles synthesis and characterization and writing the manuscript, MR was responsible for designing bacteriophage assays, carried out antibacterial tests and performed statistical analysis of the data, KK performed tests with bacteriophages and analyzed the data, SL participated in nanoparticles synthesis and writing the manuscript, MV was responsible for nanoparticles analysis, including EM imaging and elemental analysis, KS performed high resolution EM, VK and TT participated in manuscript editing.

References

1. Paintsil E, Cheng Y-C. Antiviral Agents. Encyclopedia of Microbiology [Internet]. Elsevier; 2009 [cited 2022 Jan 26]. p. 223–57. Available from: <https://linkinghub.elsevier.com/retrieve/pii/B9780123739445001784>
2. Indari O, Jakhmola S, Manivannan E, Jha HC. An Update on Antiviral Therapy Against SARS-CoV-2: How Far Have We Come? Frontiers in Pharmacology [Internet]. 2021;12. Available from: www.frontiersin.org/articles/10.3389/fphar.2021.632677
3. Liang L, Ahamed A, Ge L, Fu X, Lisak G. Advances in Antiviral Material Development. ChemPlusChem. 2020;85:2105–28.

4. Chen L, Liang J. An overview of functional nanoparticles as novel emerging antiviral therapeutic agents. *Materials Science and Engineering: C* [Internet]. 2020;112:110924. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7195146/>
5. Vecitis CD. Antiviral-nanoparticle interactions and reactions. *Environ Sci: Nano*. 2021;8:11–9.
6. Gurunathan S, Qasim M, Choi Y, Do JT, Park C, Hong K, et al. Antiviral Potential of Nanoparticles—Can Nanoparticles Fight Against Coronaviruses? *Nanomaterials*. 2020;10:1645.
7. Rashidzadeh H, Danafar H, Rahimi H, Mozafari F, Salehiabar M, Rahmati MA, et al. Nanotechnology against the novel coronavirus (severe acute respiratory syndrome coronavirus 2): diagnosis, treatment, therapy and future perspectives. *Nanomedicine*. 2021;16:497–516.
8. Maus A, Strait L, Zhu D. Nanoparticles as delivery vehicles for antiviral therapeutic drugs. *Engineered Regeneration*. 2021;2:31–46.
9. Yadavalli T, Shukla D. Role of metal and metal oxide nanoparticles as diagnostic and therapeutic tools for highly prevalent viral infections. *Nanomedicine: Nanotechnology, Biology and Medicine*. 2017;13:219–30.
10. Maduray K, Parboosing R. Metal Nanoparticles: a Promising Treatment for Viral and Arboviral Infections. *Biol Trace Elem Res*. 2021;199:3159–76.
11. Milovanovic M, Arsenijevic A, Milovanovic J, Kanjevac T, Arsenijevic N. Nanoparticles in Antiviral Therapy. *Antimicrobial Nanoarchitectonics* [Internet]. Elsevier; 2017 [cited 2022 Jan 26]. p. 383–410. Available from: <https://linkinghub.elsevier.com/retrieve/pii/B9780323527330000148>
12. Strasfeld L, Chou S. Antiviral Drug Resistance: Mechanisms and Clinical Implications. *Infectious Disease Clinics of North America*. 2010;24:413–37.
13. Rosenberg M, Ilić K, Juganson K, Ivask A, Ahonen M, Vinković Vrček I, et al. Potential ecotoxicological effects of antimicrobial surface coatings: a literature survey backed up by analysis of market reports. *PeerJ*. 2019;7:e6315.
14. Salleh A, Naomi R, Utami ND, Mohammad AW, Mahmoudi E, Mustafa N, et al. The Potential of Silver Nanoparticles for Antiviral and Antibacterial Applications: A Mechanism of Action. *Nanomaterials*. 2020;10:1566.
15. Lara HH, Ixtepan-Turrent L, Garza-Treviño EN, Rodriguez-Padilla C. PVP-coated silver nanoparticles block the transmission of cell-free and cell-associated HIV-1 in human cervical culture. *Journal of Nanobiotechnology*. 2010;8:15.
16. Lara HH, Ayala-Núñez NV, Ixtepan-Turrent L, Rodriguez-Padilla C. Mode of antiviral action of silver nanoparticles against HIV-1. *J Nanobiotechnol*. 2010;8:1.
17. Sun RWY, Chen R, Chung NPY, Ho CM, Lin CLS, Che CM. Silver nanoparticles fabricated in Hepes buffer exhibit cytoprotective activities toward HIV-1 infected cells. *Chemical Communications*. Royal Society of Chemistry; 2005;5059–61.
18. Elechiguerra JL, Burt JL, Morones JR, Camacho-Bragado A, Gao X, Lara HH, et al. Interaction of silver nanoparticles with HIV-1. *J Nanobiotechnol*. 2005;3:6.

19. Szymańska E, Orłowski P, Winnicka K, Tomaszewska E, Baska P, Celichowski G, et al. Multifunctional Tannic Acid/Silver Nanoparticle-Based Mucoadhesive Hydrogel for Improved Local Treatment of HSV Infection: In Vitro and In Vivo Studies. *IJMS*. 2018;19:387.
20. Saadh MJ, Aggag MM, Alboghdadly A, Kharshid AM, Aldalaen SM, Abdelrazek MA. Silver nanoparticles with epigallocatechingallate and zinc sulphate significantly inhibits avian influenza A virus H9N2. *Microbial Pathogenesis*. 2021;158:105071.
21. Castro-Mayorga JL, Randazzo W, Fabra MJ, Lagaron JM, Aznar R, Sánchez G. Antiviral properties of silver nanoparticles against norovirus surrogates and their efficacy in coated polyhydroxyalkanoates systems. *LWT - Food Science and Technology*. 2017;79:503–10.
22. Jeremiah SS, Miyakawa K, Morita T, Yamaoka Y, Ryo A. Potent antiviral effect of silver nanoparticles on SARS-CoV-2. *Biochemical and Biophysical Research Communications*. 2020;533:195–200.
23. Dung TTN, Nam VN, Nhan TT, Ngoc TTB, Minh LQ, Nga BTT, et al. Silver nanoparticles as potential antiviral agents against African swine fever virus. *Materials Research Express*. 2020;6:1250g9.
24. Park S, Park HH, Kim SY, Kim SJ, Woo K, Ko G. Antiviral Properties of Silver Nanoparticles on a Magnetic Hybrid Colloid. *Applied and Environmental Microbiology*. 2014;80:2343–50.
25. Rogers JV, Parkinson CV, Choi YW, Speshock JL, Hussain SM. A Preliminary Assessment of Silver Nanoparticle Inhibition of Monkeypox Virus Plaque Formation. *Nanoscale Research Letters*. 2008;3:129–33.
26. Sinclair TR, Hengel SK van den, Raza BG, Rutjes SA, Husman AM de R, Peijnenburg WJGM, et al. Surface chemistry-dependent antiviral activity of silver nanoparticles. *Nanotechnology*. 2021;32:365101.
27. Pilaquinga F, Morey J, Torres M, Seqqat R, Piña M de las N. Silver nanoparticles as a potential treatment against SARS-CoV -2: A review. *WIREs Nanomed Nanobiotechnol* [Internet]. 2021 [cited 2022 Jan 27];13. Available from: <https://onlinelibrary.wiley.com/doi/10.1002/wnan.1707>
28. Kalwar K, Shan D. Antimicrobial effect of silver nanoparticles (AgNPs) and their mechanism – a mini review. *Micro & Nano Letters*. 2018;13:277–80.
29. Ferdous Z, Nemmar A. Health Impact of Silver Nanoparticles: A Review of the Biodistribution and Toxicity Following Various Routes of Exposure. *International Journal of Molecular Sciences*. 2020;21:2375.
30. Lozovski V, Lysenko V, Piatnytsia V, Scherbakov O, Zholobak N, Spivak M. Physical Point of View for Antiviral Effect Caused by the Interaction Between the Viruses and Nanoparticles. *Journal of Bionanoscience*. 2012;6:109–12.
31. Zholobak NM, Olevinskaia ZM, Spivak NI, Shcherbakov AB, Ivanov VK, Usatenko AV. Antiviral effect of cerium dioxide nanoparticles stabilized by low-molecular polyacrylic acid. *Mikrobiolohichnyi zhurnal (Ukraine)*. 1993;72:42–7.
32. Mohamed HEA, Afridi S, Khalil AT, Ali M, Zohra T, Akhtar R, et al. Promising antiviral, antimicrobial and therapeutic properties of green nanoceria. *Nanomedicine*. 2020;15:467–88.

33. Dutta P, Pal S, Seehra MS, Shi Y, Eyring EM, Ernst RD. Concentration of Ce³⁺ and Oxygen Vacancies in Cerium Oxide Nanoparticles. *Chemistry of Materials*. 2006;18:5144–6.
34. Lyu P, Zhu J, Han C, Qiang L, Zhang L, Mei B, et al. Self-Driven Reactive Oxygen Species Generation via Interfacial Oxygen Vacancies on Carbon-Coated TiO_{2-x} with Versatile Applications. *ACS Applied Materials & Interfaces*. 2021;13:2033–43.
35. Zhang H, Qiu J, Yan B, Liu L, Chen D, Liu X. Regulation of Ce (III) / Ce (IV) ratio of cerium oxide for antibacterial application. *iScience*. 2021;24:102226.
36. Xia T, Kovochich M, Liong M, Mädler L, Gilbert B, Shi H, et al. Comparison of the Mechanism of Toxicity of Zinc Oxide and Cerium Oxide Nanoparticles Based on Dissolution and Oxidative Stress Properties. *ACS Nano*. 2008;2:2121–34.
37. Celardo I, Nicola MD, Mandoli C, Pedersen JZ, Traversa E, Ghibelli L. Ce³⁺ Ions Determine Redox-Dependent Anti-apoptotic Effect of Cerium Oxide Nanoparticles. *ACS Nano*. 2011;5:4537–49.
38. Hijaz M, Das S, Mert I, Gupta A, Al-Wahab Z, Tebbe C, et al. Folic acid tagged nanoceria as a novel therapeutic agent in ovarian cancer. *BMC Cancer*. 2016;16:220.
39. Kwon HJ, Kim D, Seo K, Kim YG, Han SI, Kang T, et al. Ceria Nanoparticle Systems for Selective Scavenging of Mitochondrial, Intracellular, and Extracellular Reactive Oxygen Species in Parkinson's Disease. *Angewandte Chemie International Edition*. 2018;57:9408–12.
40. Yang Y, Zhang M, Song H, Yu C. Silica-Based Nanoparticles for Biomedical Applications: From Nanocarriers to Biomodulators. *Accounts of Chemical Research*. 2020;53:1545–56.
41. Calvache-Muñoz J, Prado FA, Rodríguez-Páez JE. Cerium oxide nanoparticles: Synthesis, characterization and tentative mechanism of particle formation. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*. 2017;529:146–59.
42. Mohan JC, Praveen G, Chennazhi KP, Jayakumar R, Nair SV. Functionalised gold nanoparticles for selective induction of *in vitro* apoptosis among human cancer cell lines. *Journal of Experimental Nanoscience*. 2013;8:32–45.
43. Plakhova TV, Romanchuk AY, Yakunin SN, Dumas T, Demir S, Wang S, et al. Solubility of Nanocrystalline Cerium Dioxide: Experimental Data and Thermodynamic Modeling. *The Journal of Physical Chemistry C*. 2016;120:22615–26.
44. Lin Q, Lim JYC, Xue K, Yew PYM, Owh C, Chee PL, et al. Sanitizing agents for virus inactivation and disinfection. *View*. 2020;1.
45. ISO Standard 21702:2019, Measurement of antiviral activity on plastics and other non-porous surfaces [Internet]. 2019. Available from: <https://www.iso.org/standard/71365.html>
46. ISO Standard 18184:2019, Textiles — Determination of antiviral activity of textile products [Internet]. 2019. Available from: <https://www.iso.org/standard/71292.html>
47. Liu W-C, Lin S-C, Yu Y-L, Chu C-L, Wu S-C. Dendritic Cell Activation by Recombinant Hemagglutinin Proteins of H1N1 and H5N1 Influenza A Viruses. *Journal of Virology*. 2010;84:12011–7.

48. Jacobs L, de Groot R, van der Zeijst BAM, Horzinek MC, Spaan W. The nucleotide sequence of the peplomer gene of porcine transmissible gastroenteritis virus (TGEV): comparison with the sequence of the peplomer protein of feline infectious peritonitis virus (FIPV). *Virus Research*. 1987;8:363–71.
49. Carocci M, Bakkali-Kassimi L. The encephalomyocarditis virus. *Virulence*. 2012;3:351–67.
50. Chao L, Tran TT, Tran TT. The Advantage of Sex in the RNA Virus $\phi 6$. *Genetics*. 1997;147:953–9.
51. Shiba T, Suzuki Y. Localization of A protein in the RNA-A protein complex of RNA phage MS2. *Biochimica et Biophysica Acta (BBA) - Nucleic Acids and Protein Synthesis*. 1981;654:249–55.
52. Whitworth C, Mu Y, Houston H, Martinez-Smith M, Noble-Wang J, Coulliette-Salmond A, et al. Persistence of Bacteriophage Phi 6 on Porous and Nonporous Surfaces and the Potential for Its Use as an Ebola Virus or Coronavirus Surrogate. *Applied and Environmental Microbiology*. 2020;86.
53. Carvalho NA de, Stachler EN, Cimabue N, Bibby K. Evaluation of Phi6 Persistence and Suitability as an Enveloped Virus Surrogate. *Environmental Science & Technology*. 2017;51:8692–700.
54. Fedorenko A, Grinberg M, Orevi T, Kashtan N. Survival of the enveloped bacteriophage Phi6 (a surrogate for SARS-CoV-2) in evaporated saliva microdroplets deposited on glass surfaces. *Scientific Reports*. 2020;10:22419.
55. D'Souza DH, Su X. Efficacy of Chemical Treatments Against Murine Norovirus, Feline Calicivirus, and MS2 Bacteriophage. *Foodborne Pathogens and Disease*. 2010;7:319–26.
56. Kim J, Lee S, Kim C, Seo J, Park Y, Kwon D, et al. Non-monotonic concentration–response relationship of TiO₂ nanoparticles in freshwater cladocerans under environmentally relevant UV-A light. *Ecotoxicology and Environmental Safety*. 2014;101:240–7.
57. Klanjšček T, Muller EB, Holden PA, Nisbet RM. Host–Symbiont Interaction Model Explains Non-monotonic Response of Soybean Growth and Seed Production to Nano-CeO₂ Exposure. *Environmental Science & Technology*. 2017;51:4944–50.
58. Gusseme BD, Laing GD, Hennebel T, Renard P, Chidambaram D, Fitts JP, et al. Virus Removal by Biogenic Cerium. *Environ Sci Technol*. 2010;44:6350–6.
59. Neal CJ, Fox CR, Sakthivel TS, Kumar U, Fu Y, Drake C, et al. Metal-Mediated Nanoscale Cerium Oxide Inactivates Human Coronavirus and Rhinovirus by Surface Disruption. *ACS Nano*. 2021;15:14544–56.
60. FDA Code of Federal Regulations Title 21. U.S. Food & Drug Administration, 21 CFR Part 582 [Internet]. Available from: <https://www.ecfr.gov/current/title-21/chapter-I/subchapter-E/part-582>
61. FDA Code of Federal Regulations Title 21. U.S. Food & Drug Administration, 21 CFR Part 184 [Internet]. Available from: <https://www.ecfr.gov/current/title-21/chapter-I/subchapter-B/part-184>
62. Agnihothram SS, Vermudez SA, Mullis L, Townsend TA, Manjanatha MG, Azevedo MP. Silicon Dioxide Impedes Antiviral Response and Causes Genotoxic Insult During Calicivirus Replication. *Journal of Nanoscience and Nanotechnology*. 2016;16:7720–30.
63. Galdiero S, Rai M, Gade A, Falanga A, Incoronato N, Russo L, et al. Antiviral activity of mycosynthesized silver nanoparticles against herpes simplex virus and human parainfluenza virus

- type 3. International Journal of Nanomedicine. 2013;4303.
64. Mori Y, Ono T, Miyahira Y, Nguyen VQ, Matsui T, Ishihara M. Antiviral activity of silver nanoparticle/chitosan composites against H1N1 influenza A virus. *Nanoscale Research Letters*. 2013;8:93.
 65. European Chemicals Agency. Guidance on the Biocidal Products Regulation. Volume II, Efficacy. Assessment and Evaluation (Parts B+C). [Internet]. LU: Publications Office; 2018. Available from: <https://data.europa.eu/doi/10.2823/95242>
 66. Qi M, Li W, Zheng X, Li X, Sun Y, Wang Y, et al. Cerium and Its Oxidant-Based Nanomaterials for Antibacterial Applications: A State-of-the-Art Review. *Front Mater*. 2020;7:213.
 67. Hoecke KV, Quik JTK, Mankiewicz-Boczek J, Schamphelaere KACD, Elsaesser A, Meeren PV der, et al. Fate and Effects of CeO₂ Nanoparticles in Aquatic Ecotoxicity Tests. *Environmental Science & Technology*. 2009;43:4537–46.
 68. Link N, Brunner TJ, Dreesen IAJ, Stark WJ, Fussenegger M. Inorganic nanoparticles for transfection of mammalian cells and removal of viruses from aqueous solutions. *Biotechnology and Bioengineering*. 2007;98:1083–93.
 69. Farias IAP, Santos CCL dos, Sampaio FC. Antimicrobial Activity of Cerium Oxide Nanoparticles on Opportunistic Microorganisms: A Systematic Review. *BioMed Research International*. 2018;2018:1–14.
 70. Masadeh MM, Karasneh GA, Al-Akhras MA, Albiss BA, Aljarah KM, Al-azzam SI, et al. Cerium oxide and iron oxide nanoparticles abolish the antibacterial activity of ciprofloxacin against gram positive and gram negative biofilm bacteria. *Cytotechnology*. 2015;67:427–35.
 71. Ravikumar S, Gokulakrishnan R, Boomi P. In vitro antibacterial activity of the metal oxide nanoparticles against urinary tract infectious bacterial pathogens. *Asian Pacific Journal of Tropical Disease*. 2012;2:85–9.
 72. Cuahtecontzi-Delint R, Mendez-Rojas MA, Bandala ER, Quiroz MA, Recillas S, Sanchez-Salas JL. Enhanced Antibacterial Activity of CeO₂ Nanoparticles by Surfactants. *International Journal of Chemical Reactor Engineering*. 2013;11:781–5.
 73. Thill A, Zeyons O, Spalla O, Chauvat F, Rose J, Auffan M, et al. Cytotoxicity of CeO₂ Nanoparticles for *Escherichia coli*. Physico-Chemical Insight of the Cytotoxicity Mechanism. *Environmental Science & Technology*. 2006;40:6151–6.
 74. Slavin YN, Asnis J, Häfeli UO, Bach H. Metal nanoparticles: understanding the mechanisms behind antibacterial activity. *Journal of Nanobiotechnology*. 2017;15:65.
 75. Xie A, Wang S, Liu W, Zhang J, Yang Y, Han J. Rapid hydrothermal synthesis of CeO₂ nanoparticles with (220)-dominated surface and its CO catalytic performance. *Materials Research Bulletin*. 2015;62:148–52.
 76. Popov AL, Zaichkina SI, Popova NR, Rozanova OM, Romanchenko SP, Ivanova OS, et al. Radioprotective effects of ultra-small citrate-stabilized cerium oxide nanoparticles in vitro and in

vivo. RSC Adv. 2016;6:106141–9.

77. Aherne D, Ledwith DM, Gara M, Kelly JM. Optical Properties and Growth Aspects of Silver Nanoprisms Produced by a Highly Reproducible and Rapid Synthesis at Room Temperature: Silver Nanoprisms from Rapid, Reproducible, Room-Temperature Synthesis. Adv Funct Mater. 2008;18:2005–16.
78. Guo Q, Huang D, Kou X, Cao W, Li L, Ge L, et al. Synthesis of disperse amorphous SiO₂ nanoparticles via sol–gel process. Ceramics International. 2017;43:192–6.
79. Miles AA, Misra SS, Irwin JO. The estimation of the bactericidal power of the blood. Epidemiology and Infection. 1938;38:732–49.

Figures

Figure 1

HRTEM images of synthesized nanoparticles. (A) nano-CeO₂(+), (B) nano-CeO₂(-), (C) nano-Ag, (D) nano-SiO₂.

Figure 2

Characteristics (A) UV-Vis spectra and (B) infrared spectra of nano-CeO₂(+) and nano-CeO₂(-). On (A) also the UV-vis spectrum of nano-CeO₂(-) mother solution is shown.

Figure 3

The effect of nano-CeO₂(+) (A) and nano-CeO₂(-) (B) on viral plaque forming activity (log PFU/ml). Note the logarithmic x-axis. Closed symbols represent non-enveloped mammalian viruses and bacteriophages. Enveloped mammalian viruses and bacteriophages are shown with open symbols.

Figure 4

Reduction of plaque forming units (PFU/ml) due to exposure of viruses to nano-CeO₂, Ce(NO₃)₃, nanoparticles of SiO₂ and Ag during 1h. (A) coronavirus SARS-CoV-2; (B) transmissible gastroenteritis virus TGEV; (C) influenza virus A/WSN/1933; (D) picornavirus EMCV; (E) bacteriophage φ6; (F) bacteriophage MS2. Closed symbols in D and F represent non-enveloped viruses. The results of three independent experiments with standard deviation are shown. Note the logarithmic scale of y-axis. Grey

dashed line shows the limit of quantification of PFU. Concentrations of nanoparticles or chemicals are shown as comparable molar concentrations.

Figure 5

Schematics of the experiment (A), where the upper part shows SARS-CoV-2 binding to ACE2 receptor without nanoparticles and lower part demonstrates the theoretical inhibition of SARS-CoV2 binding to ACE2 receptor by of CeO₂ nanoparticles. (B) shows the effect of nano-CeO₂(+) and (C) the effect of nano-CeO₂(-) particles on binding of SARS-CoV-2 onto ACE2 receptor in an ELISA assay, measured as optical density (OD450).

Figure 6

1 h bactericidal effect of nano-CeO₂, Ce(NO₃)₃, nanoparticles of SiO₂ and Ag against Gram-negative *Escherichia coli* (A) and Gram-positive *Staphylococcus aureus* (B). Results of three independent

experiments with standard deviation are expressed as reduction of log CFU/ml. The dotted line indicates a limit of quantification.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryNefedovaetal.docx](#)
- [Graphicalabstract.png](#)