CERIA NANOPARTICLES WITH SUPERIOR ADHESION AND ANTIMICROBIAL SURFACE ACTIVITY

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BACKGROUND

1. Field

[0001] The invention relates to the general field of antibacterial compositions and in particular to highly effective irradiated cerium nanoparticle compositions for use in killing both Gram-negative and Gram-positive bacteria and viruses.

2. Background

[0002] Nosocomial or hospital acquired infections (HAIs) caused by bacteria remain a serious and global problem in human health. Indirect transmission of bacteria occurs through contamination of abiotic surfaces and is largely spread by healthcare workers as they touch multiple surfaces and patients. The longer a pathogen persists on a surface, the longer it is a source of transmission. For example, Gram-positive bacteria *Staphylococcus aureus* (*S. aureus*) can survive for months on dry surfaces and as long as 3-years on plastic, methicillin-resistant *S. aureus* (MRSA) is detectable on plastic for up to 175 days, and Gram-negative *Pseudomonas aeruginosa* (*P. aeruginosa*) can survive for up 16 months on a glass surface and 5 weeks on floors.

[0003] COVID-19 also has brought worldwide challenges to humans due to the ease of transmission of the coronavirus. Its transmission is believed to occur primarily via respiratory droplets produced by an infected person as well as by contact with a surface where a droplet containing the SARS-CoV-2 virus exists. Early studies have shown that these viruses can live between 2-3 days on most common types of surfaces. Most known available disinfectants, while able to neutralize many types of viruses, usually require a reaction time on the order of 30 seconds to 10 minutes. This can cause issues when those time scales is not practical. Additionally, current disinfectants require constant reapplication in high contact areas because they do not provide residual protection against both viruses and

bacteria.

[0004] The U.S. Center for Disease Control and Prevention reports that 1 in 31 hospital patients are currently infected with a HAI, and ~20% of patients contract at least one HAI while undergoing surgery, with direct medical costs of at least $28.4 billion annually, and an additional S12.4 billion in societal costs due to early deaths and lost productivity. Furthermore, ~120,000 *S. aureus* bloodstream infections and ~20,000 associated deaths occurred in the U.S. in 2017, while ~80,000 MRSA infections with a mortality rate of ~11,000 individuals per year, has been reported with total treatment costs for MRSA infections approximately double that of *S. aureus*. Over the past two decades, only two new classes of antibiotics (namely, oxazolidinones, and cyclic lipopeptides) have been approved for medical use. Further, and due to widespread human infection, the growing number of novel bacterial variants are emerging and remain a continual threat as do the burgeoning risks associated with antimicrobial drug resistance. As such, novel strategies that offer non-specific and broad protection are urgently needed.

[0005] Unique antibacterial nanozymes such as cerium oxide/ceria nanoparticles (CNPs), are a new generation of artificial enzymes that have received much attention. Their unique functional mechanism against pathogens is derived from quick and expedient interconversion of the oxidation state between Ce^{4+} (fully reduced) and Ce^{3+} (fully oxidized). The CNPs feature oxygen vacancies (OVs), or defects, in the lattice structure, which arise through loss of oxygen and/or electrons, when alternating between CeO_2 (Ce^{3+}) and CeO_{2-x} (Ce^{4+}) during redox reactions. The antibacterial properties of CNPs and other metal oxide-based nanomaterials are considered to be non-specific and occur *via* direct and indirect effects, where oxidative stress is considered to be the main mechanism of their antibacterial activity.

SUMMARY

[0006] Thus, there exists a need in the art for antibacterial compositions with enhanced activity against both Gram-negative and Gram-positive antibacterial pathogens such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and methicillin-resistant *S. aureus* (MRSA), and viruses such as COVID-19.

[0007] Disclosed herein are rapid and residual disinfectant compositions including irradiated, optionally metal-associated cerium oxide nanoparticles and methods of making and using the compositions. In particular embodiments, the present invention relates to irradiated CNPs and silver (Ag)-doped CNPs (AgCNPs) with superior antimicrobial properties.

[0008] Embodiments of the invention include an irradiated metal-associated cerium oxide nanoparticle (IrmCNP) comprising: cerium oxide doped with a metal, wherein the IrmCNP is irradiated so as to have a ratio of Ce3+ : Ce4+ on the surface of the nanoparticle is 60% or greater. In certain embodiments, the metal is selected from the group consisting of silver, gold, ruthenium, vanadanium, copper, titanium, nickel, platinum, titanium, tin and iron, the metal optionally being in ion form. Preferably, the metal is silver. In certain embodiments, the silver is in an amount of less than 10% by weight relative to the cerium.

[0009] In some embodiments of the invention, the IrmCNP is doped with fluorine.

[0010] In certain embodiments, the IrmCNP are less than 100 nm, less than 50 nm, less than 25 nm, less than 15 nm or less than 10 nm in size.

[0011] In certain embodiments, the IrmCNP releases H_2O_2 at a rate of at least about 1.5 to about 2 times higher than the same mCNP without irradiation.

[0012] In other aspects, the invention relates to an IrmCNP comprising: (a) dissolving cerium and silver nitrate salts; (b) oxidizing the dissolved cerium and silver salts by admixture with peroxide; (c) precipitating nanoparticles by adding ammonium hydroxide to the oxidized cerium and silver salts; (d) washing and resuspending the precipitated nanoparticles in water; (e) adding hydrogen peroxide to the resuspended nanoparticles; (f) washing the nanoparticles from (e) to remove ionized silver; and (e) irradiating the precipitated nanoparticles at a dose of 10.5 Gy. Embodiments of the invention also relate to a cerium nanoparticle made according to this method.

[0013] According to certain embodiments, the invention comprises IrmCNP compositions comprising the IrmCNPs described above and a solvent or solvent mixture excipient. Preferably, the solvent or solvent mixture excipient is selected from the group consisting of water, chloroform, methylene chloride, acetone, methyl ethyl ketone, cyclohexane, ethyl acetate, diethyl ether, lower alcohols, lower dials, THF, DMSO, DMF, and any combination thereof.

[0014] In certain embodiments, the IrmCNP composition further comprises one or more of a drying agent, an organic acid, one or more surfactants, a polymer binder, and water.

[0015] In other aspects, the invention relates to a method of disinfecting a surface, comprising contacting the surface with any of the IrmCNP compositions described above.

[0016] In other aspects, the invention relates to an article comprising any of the nanoparticles or any of the nanoparticle compositions disposed on a surface thereof.

DESCRIPTION OF THE DRAWINGS

[0017] Certain embodiments are illustrated by way of example, and not by way of limitation, in the figures of the accompanying drawings.

[0018] FIG. 1 is a model showing syntheses for AgCNP1 and AgCNP2.

[0019] FIG. 2 presents data showing the effect of X-ray irradiation on cerium nanoparticles.

[0020] FIG. 3A and FIG. 3B present data on eradication of *P. aeruginosa* by pre-irradiated CNPs (IrCNPs) and non-irradiated CNPs, respectively. FIG. 3C and FIG. 3D present data on eradication of *S. aureus* by pre-irradiated CNPs (IrCNPs) and non-irradiated CNPs, respectively. FIG. 3E and FIG. 3F present data on eradication of MRSA by pre-irradiated CNPs (IrCNPs) and non-irradiated CNPs, respectively.

[0021] FIG. 4A (IrAgCNPs) and FIG. 4B (AgCNPs) show eradication and minimum inhibitory concentrations against *P. aeruginosa* for non-irradiated AgCNPs (100 μg) and for IrAgCNPs (20 μg). FIG. 4C (IrAgCNPs) and FIG. 4D (AgCNPs) show eradication and that minimum inhibitory concentrations against *S. aureus* for non-irradiated AgCNPs and for IrAgCNPs are higher. FIG. 4E (IrAgCNPs) and FIG. 4F (AgCNPs) show eradication and minimum inhibitory concentrations against MRSA for both non-irradiated AgCNPs and for IrAgCNPs.

[0022] FIG. 5A presents data on hydrogen peroxide release from CNPs and AgCNPS versus IrCNPs and IrAgCNPs. FIG. 5B presents data on nitric oxide (NO) release and dissociates ($NO/NO^{2-}/NO^{3-}/NOx$) from CNPs and AgCNPs versus IrCNPs and IrAgCNPs.

DETAILED DESCRIPTION

1. Overview

[0023] The present disclosure relates to methods for altering the synthesis method of cerium oxide nanoparticle and metal-cerium oxide nanoparticle formulations which include exposure of the nanoparticle surface to ionizing radiation and to the superior products which are obtained. The antibacterial activity of the nanoparticles are substantially increased over that of the prior art.

2. Definitions

[0024] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. Although various

methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. However, the skilled artisan understands that the methods and materials used and described are examples and may not be the only ones suitable for use in the invention. Moreover, as measurements are subject to inherent variability, any temperature, weight, volume, time interval, pH, salinity, molarity or molality, range, concentration and any other measurements, quantities or numerical expressions given herein are intended to be approximate and not exact or critical figures unless expressly stated to the contrary.

[0025] In the foregoing specification, the invention has been described with reference to specific embodiments thereof. It will, however, be evident that various modifications and changes may be made thereto without departing from the broader spirit and scope of the invention. The specification and drawings are, accordingly, to be regarded in an illustrative rather than a restrictive sense. Throughout this specification and the claims, unless the context requires otherwise, the word "comprise" and its variations, such as "comprises" and "comprising," will be understood to imply the inclusion of a stated item, element or step or group of items, elements or steps but not the exclusion of any other item, element or step or group of items, elements or steps. Furthermore, the indefinite article "a" or "an" is meant to indicate one or more of the item, element or step modified by the article.

[0026] Unless specifically stated or obvious from context, as used herein, the term "about" is understood as within a range of normal tolerance in the art, for example within 2 standard deviations of the mean. About can be understood as within 30%, 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. Unless otherwise clear from context, all numerical values provided herein are modified by the term about. Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in specific non-limiting examples are reported as precisely as possible. Any numerical value, however, inherently contains certain errors necessarily resulting from the standard deviation found in their respective testing measurements at the time of this writing. Furthermore, unless otherwise clear from the context, a numerical value presented herein has an implied precision given by the least significant digit. Moreover, all ranges disclosed herein are to be understood to encompass any and all sub-ranges subsumed therein. For example, a range of "less than 10" can include any and all sub-ranges between (and including) the minimum value of zero and the maximum value of 10, that is, any and all sub-ranges having a minimum

value of equal to or greater than zero and a maximum value of equal to or less than 10, e.g., 1 to 4.

[0027] As used herein, the term "disinfection" or "disinfect" refers to a reduction or elimination of pathogenic microorganisms on surfaces including bacteria and viruses.

[0028] As used herein, the term "residual disinfection" as used herein refers to any sprayed disinfectant capable of disinfecting a surface for at least 24 hours in dry form. Residual disinfectants that last up to 24 hours generally disinfect 3 1og reduction of viral load and 5 1og reduction of bacterial load in under 10 minutes. Residual disinfectants (sprayed or applied by other means) that persist longer than a day generally disinfect at 3 1og reduction viral load and 3 1og reduction bacterial load within 2 hours.

[0029] As used herein, the term "rapid disinfection" as used herein refers to near instantaneous elimination of a pathogenic microorganism on surfaces. Rapid disinfectants generally have a dwell time for disinfection of about 1 minute or less when applied in wet form.

[0030] As used herein, the term "metal-associated cerium oxide nanoparticles," "metal-associated ceria nanoparticles," or "mCNPs" refers to cerium oxide nanoparticles doped with or otherwise bound to a metal such as silver, gold, copper, platinum, nickel, iron, titanium, ruthenium, vanadanium and the like. The term mCNPs includes AgCNPs. In an embodiment, the metal-associated cerium oxide nanoparticles comprise a particle size of the range of from 1nm to 50 nm or from 5 nm to 100 nm or from 5 nm to 25 nm.

[0031] As used herein, the term "nanoRAD" as used herein refers to a disinfectant with cerium oxide nanoparticles associated with a metal such as silver as the active agent, and an excipient. As taught herein, the disclosed nanoRAD compositions may include excipients such as organic acid, surfactant, drying agent and/or polymer, among others.

[0032] As used herein, the term "predominant 4+ surface charge" refers to the concentration of cerium ions on the surface and means that the [Ce3+]:[Ce4+] ratio on the surface of the cerium oxide nanoparticle is less than 50%. In a specific example, cerium oxide nanoparticles having a predominant 4+ surface charge have a [Ce3+]:[Ce4+] ratio that is 40% or less. The term "predominant 3+ surface charge" means that the [Ce3+]:[Ce4+] ratio on the surface of the cerium oxide nanoparticle is greater than 50%. In a specific example, the [Ce3+]:[Ce4+] ratio is greater than 60%. Particles having high Ce3+ surface sites relative to Ce4+" means that the [Ce3+]:[Ce4+] ratio is greater than 60%.

[0033] As used herein, the term "wet chemical synthesis" refers to a method of making CNPs that involves dissolving a cerium precursor salt in water followed by addition of hydrogen peroxide. In a specific example, the CNPs are stabilized over a predetermined time period, typically at least 15-30 days.

[0034] As used herein, the terms "CNP," refers to cerium oxide nanoparticle(s). Prefixes on this term refer to specific types of CNP. For example "mCNP" refers to CNPs doped with a metal. "IrCNP" refers to CNPs that have been irradiated. "AgCNP" refers to an mCNP where the doping metal is silver. IrmCNP refers to an irradiated mCNP. IrAgCNP refers to an irradiated AgCNP.

3. Description of Certain Embodiments

[0035] Embodiments of the invention here described relate to formulations of cerium oxide nanoparticles with higher numbers of Ce3+ sites and lower numbers of C4+ sites on the surface of the particles than those found in the prior art. The particles are formed by including an irradiation step in the production method. Ionizing radiation modifies the surface valence state of the cerium ions, thereby altering the surface kinetics and activity of the nanoparticles, and increases, for example, H_2O_2 production and potentially its synergistic interaction with nitric oxide. The irradiation treatment therefore surprisingly improves the antibacterial performance of the particles. The prior exposure of the nanoparticles to ionizing radiation improves significantly the nanoparticles' ability to eradicate both Gram-negative and Gram-positive bacteria and likely viruses such as SARS-CoV-2.

[0036] Most disinfectant sprays and other compositions only disinfect at the time of application. After application, the compositions according to embodiments of the invention create a temporary, continually disinfecting film left behind on the surface to which it is applied. This persistent, disinfectant activity is due to the regenerative (catalytic) properties of the ceria nanoparticles (CNP) nano-surface reaction sites which allow for continued disinfection of a surface when new viruses or bacteria come into contact with it. Thus, compositions containing the inventive nanoparticles can curb transmission of COVID 19 and Hospital Acquired Infections (HAis) in a highly effective manner by producing a temporary, continually disinfecting film.

[0037] Presently, no agent exists that can efficiently reduce the spread of community, and hospital associated microorganisms. The manufacturing scale up of IrCNPs is possible, and the information in this invention not only supports the feasibility of using IrCNPs as a broad-

spectrum antimicrobial but may also be used to develop custom approaches too. When CNP are irradiated according to certain embodiments of the invention, their antimicrobial properties and potency are improved.

[0038] This means that the potency of the nanoparticles is improved, and the effective concentration and dose of nanoparticles needed to eradicate bacteria, is lower. A second advantage to the inventive irradiated CNPs is in the hydrogen peroxide /NO synergistic coupling that occurs. This nanomaterial-based activity when in lower concentrations, can offer a new antimicrobial strategy for many applications in vivo too. This present disclosure allows one to engineer the nanomaterial's antimicrobial activity more precisely, and towards increasing the targeted activity (through improved adhesion), as well as delivering a unique combination of chemical activity towards microorganisms on an abiotic surface, and/or host cells within the body.

[0039] Thus, in summary, the irradiated CNPs according to embodiments of the present disclosure are surprisingly more effective compared to previous such products due to the structural surface modifications produce during irradiation.

A. Nanoparticles

[0040] Nanoparticles according to the present disclosure are both rapid acting and residual acting compositions for disinfection, which are useful to curb the transmission of SARS-CoV-2, and other viruses, in addition to bacterial species, via contact with surfaces. The nanoparticles perform several 'disinfectant' reactions in parallel involving cerium oxide surface reactions and are improved in function over the prior art particles. These mechanisms are self-regenerating since the nanoparticles are not used up in the disinfection process, allowing them to have residual disinfection capabilities.

[0041] These nanoparticles have an increased ratio of Ce3+ versus Ce4+ sites on their surface of 60% or greater.

[0042] In some embodiments, the particles can incorporate one or more metals, such as silver, leading to further generation of free radicals in application. The metal associated with the cerium oxide nanoparticles includes but is not limited to silver, gold, ruthenium, vanadanium, copper, titanium, nickel, platinum, titanium, tin and iron. Transition metal-based materials have shown exceptional broad- spectrum anti-bacterial activity as well as anti-viral efficacy. Preferably, the metal is silver and comprises 10% or less of the weight of the particle.

[0043] Doping of the nanoparticles (nanoceria) with fluorine, or similar chemistry, also can be done to decrease the reaction rate of the first two mechanisms, to well below 30 seconds. The combination of disinfecting mechanisms, working together, reduces the overall rate event further, allowing for rapid disinfection by multiple concurrent routes, and dry disinfecting potency at concentrations that are safe for contact.

[0044] The mCNP may be spherical, rod-shaped, star-shaped, or polygonal. In a preferred embodiment, the mCNP are spherically-shaped, meaning that they more or less approximate the shape of a sphere. Preferably, the average diameter of the spherically-shaped mCNP is about 24 nm or less, about 20 nm to about 24 nm or about 3 nm to about 5 nm. In a certain embodiment, the spherically-shaped cerium oxide nanoparticles have an average diameter of 3 nm to 5 nm as measured by transmission electron microscopy. In embodiments in which the mCNP are not spherically shaped, it is preferred that the average dimension between two opposing sides of the nanoparticles is 24 nm or less.

[0045] The CNP have a cerium oxide core with an external surface. The surface is characterized according to the ratio of the percentage of Ce(3+) relative to Ce(4+) ions thereon. The irradiation step improves this ratio to increase Ce(3+) relative to Ce(4+) and produce a superior product with increased disinfection properties. Thus, the irradiated CNP according to embodiments of the present disclosure have a Ce(3+):Ce(4+) percentage ratio of about 60%:40% or greater, and optionally about 62%:38% or greater or about 65%:35% or greater, or about 70%:30% or greater. The structural and functional properties of CNP formulations according to the present disclosure compared to those without an irradiation step as described herein are shown in Table 1, below, in Example 6.

[0046] The embodiments disclosed here has involved developing a silver-ceria nanoparticle (AgCNP) and ceria (CNP) formulation with superior levels of Ce^{3+} surface sites relative to Ce^{4+}, for use as a potent antimicrobial agent for hard surfaces. Through engineering the surface, and thus degree of electron exchange, nanoparticle adhesion and interaction to/with the microorganism/s can be increased or decreased, thereby regulating the antimicrobial effect. Surprisingly, the antimicrobial effects are driven by the direct surface synthesis of hydrogen peroxide combined with nitric oxide (NO) and/or its dissociates (*e.g.*, NO/NO_2^-/NO_3^-/NOx), and this activity is non-photocatalytic. Without wishing to be bound by theory, hydrogen peroxide and NO aid in eradicating microorganisms, while their synergistic combination on the nanosurface, forms secondary harmful radicals and non-radicals, able to sustain/augment the antimicrobial effect.

[0047] By altering the synthetic process to include exposure of the CNP to ionizing radiation (X-ray), the surface Ce^{3+} and Ce^{4+} valence distributions are modified in a time dependent manner. Radiation exposure increases the antibacterial potency of both CNPs and AgCNPs. This increased activity is, at least in part, due to the increased Ce^{3+} surface activity, and thus increased formation of hydrogen peroxide, and additionally the production of nitric oxide (NO) and/or its dissociates (*e.g.*, NO/NO_2^-/NO_3^-/NOx). Both hydrogen peroxide and NO independently aid in eradicating microorganisms, and further, their synergistic combination forms important secondary harmful radicals and non-radicals (e.g., singlet oxygen), that are also able to sustain/augment the antimicrobial effect. As the activity appears time- and potentially dose-dependent, it is conceivable that nanoparticle exposure to non-ionizing (e.g., microwave), and ionizing, e.g., gamma rays, alpha particles, beta particles, and positrons, delivered using equipment on Earth, as well as the complex mix of X-rays, gamma rays, and streams of protons and electrons from solar particle events and galactic cosmic radiation, among other forms of highly ionized particles found within the space environment (both near orbit and farther afield), will also further alter both CNP and AgCNP surface valence sites etc. and subsequent biological activity, including its antibacterial activity.

[0048] According to one embodiment, the present disclosure relates to a dispensable composition including a metal-associated cerium oxide nanoparticles (IrmCNP) as described herein and an excipient. In some embodiments, the excipient is selected from the group consisting of

water, chloroform, methylene chloride, acetone, methyl ethyl ketone, cyclohexane, ethyl acetate, diethyl ether, lower alcohols, lower dials, THF, DMSO, or DMF.

B. Production

[0049] In certain embodiments, the present disclosure also relates to a process for making the nanoparticles. See FIG. 1 for a general diagram. Cerium oxide nanoparticles are produced according to any method known in the art. For example, for AgCNPs, the nanoparticles can be produced via a general method comprising dissolving cerium and silver precursor salts such as cerium and silver nitrates; oxidizing the dissolved cerium and silver precursor salts via admixture with peroxide; and precipitating nanoparticles by subjecting the admixture with ammonium hydroxide.

[0050] Alternatively, the IrAgCNPs are produced via a method comprising (i) dissolving

cerium and silver precursor salts such as cerium and silver nitrates; (ii) oxidizing and precipitating the dissolved cerium and silver precursor salts via admixture with ammonium hydroxide; (iii) washing and resuspending precipitated nanoparticles in water; (iv) subjecting the resuspended nanoparticles with hydrogen peroxide; and (v) washing the nanoparticles from step (iv) to remove ionized silver. Prior to use, the particles are irradiated as follows: irradiation was performed at a distance of 30 cm from the nanoparticles. The radiation dose can be increased or decreased (by altering the time of exposure). For example, a radiation dose of about 1 Gy to about 15 Gy or more is generally suitable. Preferably, the radiation dose is about 10 Gy to about 13 Gy or about 10.5 Gy to about 12 Gy. For example, a dose of 10.5 Gy is preferable. Without wishing to be bound by theory, higher doses are most preferred in order to alter the Ce3+ and Ce4+ ratio and deliver increased levels of H_2O_2 and nitric oxide (NO) and/or its dissociates (*e.g.*, NO/NO_2^-/NO_3^-/NOx).

[0051] Metal associated cerium oxide nanoparticles also can be made according to methods previously disclosed. Preferred silver-associated cerium oxide nanoparticles are made in two different types, which can be used separately or in combination. Preferred silver modified cerium oxide formulations (AgCNPs) can synthesized in two unique formulations (AgCNP1, AgCNP2) each utilizing different chemical reactions specific to aqueous silver. AgCNP1 is synthesized via a previously developed, two step procedure (see Example 5) that can be scaled to a large or small process.

[0052] There are several methods to synthesize nanoceria particles, including wet chemical, solvothermal, microemulsion, precipitation, hydrolysis and hydrothermal. Based on the synthesis methodology employed, the size of these NPs varies broadly from 3-5 nm to over 100 nm, and the surface charge can vary from -57 mV to +45 mV. The synthesis method can also affect the shape of CNPs. In general, wet chemical methods are preferred and potentially can produce a product with less toxicity.

[0053] In some embodiments, the two formulations of silver-associated cerium oxide nanoparticles carry silver nano-phase modified surfaces. Materials characterization has shown that the silver components in each of the two formulations are different from each other and decorate the ceria surface as many small nanocrystals (AgCNP1) or as a Janus-type two-phase construct (AgCNP2).

[0054] Preferably, the average diameter of AgCNP1 is about 20 to 24 nm, and the average diameter of AgCNP2 is about 3 to 5 nm. Each synthesis further possesses different mixed valency with AgCNP2 possessing a significantly greater fraction of Ce3+ states relative to

Ce4+ over AgCNP2 even prior to irradiation. The distinct valence characteristics, along with incorporation of chemically active silver phases, leads to high catalytic activities for each formulation. AgCNP2 possesses high superoxide dismutase activity, while AgCNP1 possesses both catalase and superoxide dismutase-like enzyme- mimetic activities, ascribed to the catalase activity of ceria and the superoxide dismutase activity from silver phases.

[0055] Further, electrochemical analysis demonstrated that the silver incorporated in each formulation is substantially more stable to redox-mediated degradation than pure silver phases: promoting an increased lifetime in catalytic applications. Use of each formulation in effecting anti-viral properties showed a specific activity for each formulation: with, among the virus species tested, AgCNP1 showing substantial activity towards OC43 coronavirus and AgCNP2 active against RV14 rhinovirus. In situ electrochemical impedance spectra collected for each virus/particle system over the respective incubation periods mirrored the unique interactions observed for each pairing. Equivalent circuit fittings for each, along with developed model/test systems (use of analog virus-like particles, model protein, radical oxygen species generating enzyme/substrate systems), showed the modes of action for the pairings in effecting anti-viral responses. The results of these investigations assign a dominate physical interaction-based mechanism for OC43/AgCNP1 while an oxidative, chemical interaction is determined for RV14/AgCNP2.

[0056] Although the amount is not intended to be limiting, when used in methods of the present disclosure, some preferred amounts of silver percentages associated with the AgCNPs are about 6% to about 10%, or less.

[0057] In the irradiation step of the production process, the nanoparticles were exposed to controlled levels of ionizing (X-ray) radiation at a dose of 10.5 Gy and at a distance of 30 cm from the radiation source.

[0058] A preferred embodiment of the present disclosure is a synthetic method where a silver-modified formulation of CNPs (AgCNPs) synthesized at a cerium:silver molar ratio of 5:1, is formulated as follows: cerium nitrate hexahydrate (99.999% purity) was used as a cerium source and dissolved to 5.0 mM (final concentration) in de-ionized water (dH_2O) (>20 MΩ). For the AgCNP formulation, silver nitrate (99.9% purity) was added subsequently at 1.0 mM (final concentration). The product materials were washed with dH_2O and subsequently treated with ammonium hydroxide, which mediated aqueous dispersion of dissolved silver ($Ag[(NH_3)_2OH]_{aq}$), followed by another washing step. From here, 2.0 mL of 3.0% hydrogen peroxide was added such that the final solution volume was 50 mL and prior

to immediate mixing *via* vortexing. Solutions were then allowed to age (until samples become clear and well-dispersed) in dark conditions, with storage bottle caps loosened to allow release of evolved gases and maintenance of standard pressure conditions. The nanoparticles then are subjected to irradiation as follows: AgCNPs, CNPs, and water were subjected to 10.5 Gy of irradiation, which was applied using a 160 kV tube voltage, 4 mA tube current, and a distance of 30 cm between the source and the nanoparticles (SC 500 smart controller, KIMTRON, USA).

C. Formulations

[0059] In certain embodiments, disclosed is a dispensable composition comprising CNPs (e.g. IrAgCNPs) and an excipient. Examples of excipients include solvents, preferably liquid solvents, such as but are not limited to, water or water-based (aqueous) solutions in which water is at least the main component, lower alcohols (C6 or lower), lower dials (C6 or lower), tetrahydrofuran (THF), dimethyl sulfoxide (DMSO), dimethylformamide (DMF), and the like. Solvents can be used alone or as mixtures of various components with water. Non-limiting examples of nonaqueous carriers or mixtures thereof include chloroform, methylene chloride, acetone, methyl ethyl ketone, cyclohexane, ethyl acetate, diethyl ether, lower alcohols (C4 or less), lower dials (C4 or less), THF, DMSO, DMF, and any combination thereof.

[0060] The dispensable composition optionally also comprises a fragrance. Examples of fragrance include, but are not limited to, lemon oil, orange oil, bergamot oil, ylang ylang oil, patchouli oil, citronella oil, lemongrass oil, boad rose oil, clove oil, eucalyptus oil, cedar oil, lavender oil, natural fragrances such as sandalwood oil, vetiver oil, geranium oil, labdanum oil, peppermint oil, rose oil, jasmine oil, litz accubeba oil; hydrocarbon- based fragrances (e.g. limonene, a-pinene, camphene, p-cymene, phen Chen, etc.), ether perfumes (for example, 1,8-cineole, rose oxide, cedrol methyl ether (cedlum bar), p-cresyl methyl ether, isoamylphenyl ethyl ether, 4-phenyl-2,4,6-trimethyl-1,3-dioxane, anethole, etc.), S Perfume (for example, ethyl acetate, ethyl propionate, methyl butyrate, ethyl isobutyrate, ethyl butyrate, butyl acetate, ethyl 2-methylbutyrate, isoamyl acetate, ethyl 2-methylpentanoate (manzanate), hexyl acetate, allyl hexanoate, tricyclodecenyl propionate (VERTOPRO; fluorocyclene), allyl heptanoate, isobornyl acetate, linalyl acetate, citronellyl acetate, 2-ter-butylcyclohexyl acetate (narcidol) etc.), alcoholic fragrances (e.g., linanol, 3-octanol, 2,6-dimethyl-heptanol, 10-undecenol, geraniol, nerol, citronellol, rosinol, mill Senol, tetrahydrolinalol, thymol, terpineol, cedrol, 2,4-dimethyl-3-cyclohexane-1-methanol, 4-

isopropylcyclohexanol, nerolidol, 9-decenol, cis-3-hexenol, trans-2-hexenol, eugenol, etc.), Aldehyde perfume (for example, citronella, para aldehyde, benzaldehyde, aldehyde C-6, aldehyde C-7, aldehyde C-8, aldehyde C-9, aldehyde C-10, tripral, p-ethyldimethylhydrocinnamic aldehyde), synthetic fragrances such as (florazone), 2-tridecenal, aldehyde C11, etc.) or blended fragrances comprising one or more of the above. In other embodiments, the embodiments can provide antipathogen foams which can be used for a number of applications. For example, polyurethane foams are made using a formulation produced by mixing an isocyanate with a polyol (a molecule with three or more hydroxyl groups) a chain extender (a bifunctional hydroxyl molecule), catalysts to promote reaction, surfactant, heat and/or UV stabilizers along with a foaming agent. The foaming agent could be water as it produces carbon dioxide gas when it reacts with the isocyanate. One method of making antiviral foams involves producing metal-associated cerium oxide nanoparticles with a surfactant (using a surfactant compatible with the system or the same which is used in the system) or one of the urethane-forming constituents and adding these to the foam formulation, before or after irradiation of the CNPs. Another alternative involves producing nanoparticles in an aqueous media, such as by mixing them in water along with the desired surfactant and then adding this aqueous mixture to the foam formulation both as a foaming agent and as an antipathogen source.

[0061] The present disclosure also includes spray formulations. In typical such embodiments, the formulations comprise one or moretype of IrCNP, a drying agent, an organic acid, surfactants, water, and a polymer binder. The spray formulation preferably creates a disinfecting film when applied to a substrate. In certain embodiments, the IrCNP is in an amount ranging from about 0.01 to 10% by weight. In certain embodiments, a drying agent, such as ethanol or isopropanol, is in an amount ranging from about 0 to 40% by weight. In certain embodiments, about 0.5 to 2% citric acid, or other organic acids, by weight is provided to the spray formulation. The spray formulation compositions can be made as described in United States Patent Publication Nos. US2023-0157299 and US2023-0137248.

[0062] In certain embodiments, the spray formulations upon application create a film that can be rehydrated and shows potential continued disinfecting behavior upon re-hydration. IrAgCNPs can pull water from gaseous water particles for reactivation, and the polymer film created from the spray formulation is also hydrophilic which assists in achieving a surface water layer from gaseous water particles for reactivation of disinfecting behavior.

[0063] According to other embodiments, antiviral inks comprising irradiated cerium oxide nanoparticles associated with silver or another metal may be formed using techniques known in the art of printing inks. Such inks may be printed using a variety of techniques such as inkjet, flexo, gravure and silk-screening. In some cases, such as in inkjet printing, the size of the functionalized particles should be smaller than about 50 nm. Three dimensional antiviral products (mask material and hard objects commonly touched) may be formed by 3-D printing, where the 3-D printing compositions incorporate the antiviral materials taught herein such as IrAgCNPs.

D. Pathogens and Other Cells

[0064] Certain embodiments disclosed herein are contemplated for use in killing bacteria, viruses, and other cells on surfaces. For example, Gram-negative bacteria such as *P. aeruginosa*, *Escherichia coli*, *Salmonella enterica*, and *Acinetobacter* species, and the like are contemplated for eradication by embodiment of the present disclosure. Also, Gram-positive *Staphylococci* bacteria such as. *S. aureus* (including MRSA), *Streptococci*, and *Enterococci* species, and the like are contemplated for eradication by embodiments of the present disclosure. In addition, viruses can be eradicated by embodiments of the products and compositions of the present disclosure.

[0065] While non-toxic to neutral pH normo-typic mammalian cells, products and compositions according to embodiments of the present disclosure are effective in killing cancer cells due to the acidic chemical environment and nanoceria's pH-sensitive redox activity. The CNPs according to the present disclosure have been observed to display the protective effect that has been previously reported for CNPs. Therefore, embodiments are contemplated for use in killing cells such as triple negative breast cancer, osteosarcoma, and metastatic prostate cancer cells.

E. Method of Use

[0066] In further embodiments, the present disclosure relates to a method of disinfecting a surface by dispensing a dispensable composition embodiment onto the surface to be disinfected. The substrate may take the form of any surface upon which human contact is made or human expired droplets are commonly disposed. Substrates contemplated to be disinfected with the compositions of the present disclosure include, but are not limited to: metal, glass, plastic, wood, painted surfaces, fabrics, ceramics, resins, fiber, and the like. Embodiments of the invention are contemplated for use in a variety of locations, including hospitals and other medical facilities; long-term care and nursing home facilities; schools;

prisons; food distribution centers; meat packing plants; restaurant kitchens; public areas such as amusement parks, museums, theaters and the like; public conveyances such as airplanes, ships, trains, buses, subways, and the like; aerospace applications such as space stations and space capsules; and in the home.

[0067] Specific examples of suitable substrates for use with embodiments of the invention include, but are not limited to home and hospital products (such as tissues, tissue paper, paper and cloth towels, bedding, food and drink containers, childrens' toys, and the like), household and hospital surfaces and equipment (such as countertops and benches, HVAC filters, air cleaning devices, electric fans, refrigerators, microwave ovens, dish washer/driers, food preparation vessels, rice cookers, pots and pand, lids, IH heaters, washing machines, vacuum cleaners, lighting apparatuses (lamps, apparatus bodies, shades, and the like), sanitary products, toilets, washbowls, mirrors, bathrooms (walls, ceilings, floors, and the like), building materials (interior walls, ceiling materials, floors, exterior walls, and the like), interior products (curtains, carpets, tables, chairs, sofas, shelves, beds, beddings, and the like), glasses, sashes, hand rails, doors, knobs, light switches, clothes, filters used for home electric appliances or the like, stationery and writing instruments, kitchen utensils, medical supplies (white coats, masks, gloves, PPE, and the like), medical appliances and devices, and materials used inside automobiles, vehicles of trains, aircrafts, boats and ships, and the like.

F. Summary

[0068] Irradiation of CNPs specifically modifies the surface Ce3+/Ce4+ ratio of the CNP, resulting in a surprising improvement in their function. The IrAgCNP act via the preferential electrostatic adherence of anionic bacteria to the cationic surface, and the predominant non-photocatalytic, and direct synthesis of H_2O_2, and NO on the nanomaterial surface. Subsequent superior ROS formation and dysregulation, DNA damage, and cytotoxicity, leads to a catastrophic loss in both aerobic and anaerobic respiration, osmodysregulation, and finally, complete cell burst. Our results reveal several mechanistic avenues of efficacy. The potent bactericidal activity is potentially driven through Ag augmented electron transfer from the E_c to O_2, the subsequent selective reduction of O_2 to H_2O_2 formation, likely facilitated by a weakened Ag-induced Ce-O bond, the presence of increased Ce^{3+} surface sites, and the combined nanomaterial surface generation of H_2O_2 and/or $NO/NO_2^-/NO_3^-/NOx$.

5. Examples

[0069] This disclosure is not limited to the particular processes, compositions, or methodologies described, as these may vary. The terminology used in the description is for the purpose of describing the particular versions or embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the present invention, the preferred methods, devices, and materials are now described. All publications mentioned herein, are incorporated by reference in their entirety; nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

[0070] General Methods.

[0071] A. AgCNP and CNP syntheses

[0072] Both CNP and AgCNP were synthesized as follows. For both CNP and AgCNP, cerium nitrate hexahydrate (99.999% purity, Millipore Sigma™) was used as a cerium source and dissolved to 5.0 mM (final concentration) in de-ionized water (>20 MΩ). For the AgCNP formulation, silver nitrate (99.9% purity, Aldrich Chemical Company™) was subsequently added at 1.0 mM (final concentration). To this solution, 2.0 mL of 3.0% hydrogen peroxide (Millipore Sigma™) was added such that the final solution volume was 50 mL with immediate mixing by vortexing. These mixed solutions were then allowed to age (until samples become clear and well-dispersed) in dark conditions, in storage bottles with the caps loosened to allow release of evolved gases and maintenance of standard pressure conditions.

[0073] B. Bacterial strains

[0074] *Pseudomonas aeruginosa* (*P. aeruginosa*, ATCC 15442), *Staphylococcus aureus* (*S. aureus*, ATCC 6538), and methicillin resistant *Staphylococcus aureus* (MRSA, ATCC 33592) were procured from the American Type Culture Collection (ATCC, Rockville, MD, USA). All bacterial strains were cultured in Luria-Bertani (LB) (Sigma™) at 37°C and 200 rpm of agitation.

[0075] C. Determination of minimum inhibitory concentration (MIC)

[0076] The minimum inhibitory concentration (MIC), defined as the lowest concentration of a compound that inhibits the growth of an organism, was determined for *P. aeruginosa*, *S.*

aureus and MRSA in LB medium at pH 7.2. The bacterial inoculums were adjusted to a concentration of 10^8 CFU/mL. To determine the MIC of AgCNP and CNP, broth dilution tests were conducted using a 96-well flat bottom microtiter plate. Final AgCNP and CNP concentrations of 20, 40, 60, 80, 100, 200, 400 and 600 µg/mL were prepared. Cultures were grown at 37°C for 24 hours and changes in optical density (600 nm) were used to assess bacterial growth. Additionally, and following 24 hours of incubation, 100 µL of each cultured suspension was plated over LB agar, incubated overnight at 37°C, and the concentration at which total inhibition of bacterial growth occurred was recorded.

[0077] D. Bacteriostatic analysis

[0078] Bacterial inoculums were adjusted to 10^8 CFU/mL. The MIC of AgCNP (*i.e.,* 100 µg/mL for *P. aeruginosa* and 300 µg/mL for *S. aureus* and MRSA) in a final volume of 25 mL have been confirmed. LB medium inoculated with 10^8 CFU/mL (only) was used as a control. Cultures were incubated at 37°C with 200 rpm of agitation. Cultures (50 µL) were spread over LB agar plates. The number of colonies formed was counted (CFU/mL) after incubation at 37°C for 16 hours.

[0079] Studies investigated AgCNPs at their MIC, and *P. aeruginosa* at a shorter timeframe compared to *S. aureus* and MRSA. Gentamicin and $AgNO_3$ was investigated at 20 µg/mL throughout.

[0080] J. H_2O_2, and NO activity in the absence of bacteria

[0081] Levels of H_2O_2 activity were quantified using a *Pierce™ Quantitative Peroxide Assay Kit* (Molecular Probes, Thermo Fisher Scientific™). The assay was performed at a sample:working reagent ratio of 1:2. The solution was gently mixed and incubated for 15-20 minutes at room temperature, and absorbance measured at 595 nm (Spectramax iD5 multimode microplate reader, Molecular Devices™). The concentration of peroxide was calculated by reference to its assay absorbance and using a standard curve. Control samples contained distilled water only. The generation of NO was quantified using the Griess Reagent Assay (Invitrogen™). 1 mL of each sample was incubated with 100 µL Griess reagent [1:1 ratio of 1% sulfanilamide in 10% orthophosphoric acid and 0.2% napthylethylenediamine in dH2O] for 15 minutes, in the dark, and at room temperature. Absorbance was measured at 548 nm relative to the reference sample (Spectramax iD3 multimode microplate reader, Molecular Devices™).

[0082] Q. RNA-Seq Library Preparation

[0083] RNA was collected from MRSA after treatment with AgCNP, CNP, and $AgNO_3$ for 150 mins at 37°C using Direct-Zol RNA mini prep (Cat # R2050). Untreated cells served as control. Libraries were constructed and prepared using the Zymo-Seq RiboFree Total RNA Library Prep Kit (Cat # R3000, Zymo Research™). RNA-Seq libraries were sequenced using Illumina™ NovaSeq to a sequencing depth of at least 30 million read pairs (150 bp paired-end sequencing) per sample. Samples were analyzed by Zymo Research™ (Total RNA-Seq Service™).

[0084] R. RNA-Seq Data Bioinformatics Analysis

[0085] The Zymo Research RNA-Seq pipeline was originally adapted from the nf-core/rnaseq pipeline (v1.4.2), and built using Nextflow. Quality control of raw reads was carried out using FastQC (v0.11.9). Adapter and low-quality sequences were trimmed using Trim Galore™ (v0.6.6). Trimmed reads were aligned to the reference genome using STAR v2.6.1d[137]. BAM file filtering and indexing was carried out using SAMtools (v1.9). RNAseq library quality control was implemented using RSeQC (v4.0.0) and QualiMap (v2.2.2-dev). Duplicate reads were marked using Picard tools (v2.23.9). Library complexity was estimated using Preseq (v2.0.3). Duplication rate quality control was performed using dupRadar (v1.18.0). Reads overlapping with exons were assigned to genes using feature Counts (v2.0.1). Classification of rRNA genes/exons and their reads were based on annotations and RepeatMasker™ rRNA tracks from UCSC genome browser when applicable. Differential gene expression analysis was completed using DESeq2 (v1.28.0). Functional enrichment analysis was achieved using g:Profiler python API (v1.0.0). Quality control and analysis results plots were visualized using MultiQC (v1.9). A cutoff of 1.5 log fold change and a $p < 0.05$ was used to identify differentially regulated genes. A heat map was generated for differentially regulated genes to represent significant gene expression patterns. Genes were classified based on cellular function and pathways using the functional annotation tool in DAVID (Database for Annotation, Visualization and Integrated Discovery). KEGG and DAVID databases were used to identify the pathways and gene function. The significance of the pathway classification was calculated based on the EASE score. The pathways obtained were sorted based on the number of genes in a particular pathway from the DESeq data. The pathways were further classified based on cellular functions of interest.

[0086] S. Statistical Analysis

[0087] Statistical analysis was performed using a two-way ANOVA with a post-hoc Tukey's test for comparison between groups (GraphPAD™ Prism, v8, USA). p values <0.05

were considered significant. Mean values ± SE are presented. All experiments were carried out in triplicate.

[0088] Example 1: Ionizing Radiation Effects on Cerium Nanoparticles (CPNs).

[0089] Ionizing radiation modifies the surface valence state of cerium on the particle surface, thereby altering the surface kinetics and activity of CNPs. High resolution photoemission spectra of the Ce 3d region is shown in FIG. 2 (sample ST-2 CeO_2, particle sizes 3-4 nm).

The data show that the amount of Ce^{+3} increases with x-ray exposure time for 3 nm particles. A dose-response is seen where increased exposure = increased Ce^{3+}. As shown in FIG. 2 also, ionizing radiation modifies the surface valance state, thereby altering the surface kinetics and activity of the AgCNPs.

[0090] For production of the irradiated particles, previously produced particle, (AgCNPs or CNPs) and water were subjected to 10.5 Gy of radiation, which was applied using a 160 kV tube voltage, 4mA tube current, at a distance of 30 cm between the source and the surface (SC 500 smart controller, KIMTRON, USA). After irradiation, the minimum inhibitory concentration (MIC) of IrAgCNP and IrCNP was determined. The lowest concentration of IrAgCNP and IrCNP that inhibits the growth of an organism was determined for *P. aeruginosa* (ATCC 15442), *S. aureus* (ATCC 6538) and methicillin resistant *S. aureus* (MRSA, ATCC 33592) in Luria Broth (LB) medium at pH 7.2. The bacterial inoculums were adjusted to a concentration of 10^8 CFU/mL. LB medium inoculated with 10^8 CFU/mL (only) was used as a control. An equal volume of irradiated water (Irwater) was also investigated as a control for the assay.

[0091] To determine the MIC of IrAgCNPs, broth dilution tests were conducted using a 96-well flat bottom microtiter plate. Final IrAgCNP and IrCNP concentrations of 20, 40, 60, 80, 100, 200, 400 and 600 µg/mL were prepared for *S. aureus* and MRSA. Final concentrations of 5, 10, 15, 20, 40, 60, 80, 100, 200, 400 and 600 µg/mL were prepared for *P. aeruginosa.* Cultures were grown at 37°C for 24 hours and changes in optical density (610 nm) were used to assess bacterial growth. Additionally, and following 24 hours of incubation, 5 µL of each cultured suspension was plated over LB agar, incubated overnight at 37°C, and the concentration at which total inhibition of bacterial growth occurred was recorded.

[0092] Data is presented as mean ± standard error. Experiments were carried out in triplicate. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$.

[0093] Example 2: Comparative Effectiveness of Irradiated CNPs.

[0094] Non-irradiated and irradiated CNPs were tested against P. aeruginosa, S. aureus, and MRSA, as shown in FIG. 3.

[0095] A culture of either *Pseudomonas aeruginosa*, *Staphylococcus aureus* or methicillin-resistant *Staphylococcus aureus* (MRSA) were prepared. After irradiation, the minimum inhibitory concentration (MIC) of IrAgCNP and IrCNP was determined. The lowest concentration of IrAgCNP and IrCNP that inhibits the growth of an organism, was determined for *P. aeruginosa* (ATCC 15442), *S. aureus* (ATCC 6538) and MRSA (ATCC 33592) in Luria Broth (LB) medium at pH 7.2. The bacterial inoculums were adjusted to a concentration of 10^8 CFU/mL. LB medium inoculated with 10^8 CFU/mL (only) was used as a control. An equal volume of irradiated water (Irwater) was also investigated as a control for the assay. To determine the MIC of IrAgCNPs, broth dilution tests were conducted using a 96-well flat bottom microtiter plate. Final IrAgCNP and IrCNP concentrations of 20, 40, 60, 80, 100, 200, 400 and 600 µg/mL were prepared for *S. aureus* and MRSA. Final concentrations of 5, 10, 15, 20, 40, 60, 80, 100, 200, 400 and 600 µg/mL were prepared for *P. aeruginosa.* Cultures were grown at 37°C for 24 hours and changes in optical density (610 nm) were used to assess bacterial growth. Additionally, and following 24 hours of incubation, 5 µL of each cultured suspension was plated over LB agar, incubated overnight at 37°C, and the concentration at which total inhibition of bacterial growth occurred was recorded.

[0096] Testing of CNPs versus IrCNPs revealed the significant inhibition of *P. aeruginosa,* in the IrCNP group. See FIG. 3A. Without irradiation, CNPs were unable to substantially eradicate *P. aeruginosa*. See FIG. 3B. Testing against *S. aureus* showed the CNPs were unable to reduce bacterial growth enough to reach the minimal inhibitory concentration (MIC). See FIG. 3D. However, the exposure of *S. aureus* to IrCNPs significantly reduced their growth and revealed a MIC of 400 µg, confirming their complete eradication at this concentration. See FIG. 3C. Testing against MRSA growth also showed the CNPs were unable to reduce bacterial growth enough to reach the MIC. See FIG. 3F. In contrast, exposure to IrCNPs significantly reduced MRSA growth, with an MIC measured at 600 µg. See FIG. 3E.

[0097] Against *P. aeruginosa*, non-irradiated CNPS do not completely inhibit bacteria even at the highest concentration of 600 µg/m, whereas the irradiated CNPs effectively inhibited *P.*

aeruginosa at a concentration beginning at (MIC) 300 µg/mL. See FIG. 3A and FIG. 3B. Against *S. aureus*, non-irradiated CNPs did not completely inhibit *S. aureus* even at concentrations up to 600 µg/mL, whereas the irradiated CNPs effectively inhibited *S. aureus* at a concentration beginning at (MIC) 400 µg/mL. See FIG. 3C and FIG. 3D. Against MRSA, non-irradiated CNPs do not completely inhibit MRSA at concentrations up to 600 µg/mL, whereas the irradiated CNPs effectively inhibited MRSA at a concentration beginning at (MIC) 600 µg/mL.

[0098] Thus, prior exposure of CNPs to ionizing radiation, followed by their application to bacteria, significantly improved the nanoparticle's ability to eradicate both Gram-negative and Gram-positive bacteria.

[0099] Example 3: Minimum Inhibitory Concentrations (MIC) of AgCNPs.

[0100] The minimum inhibitory concentrations (all growth stopped) of tested nanoparticles were calculated for irradiated (Ir) and non-irradiated AgCNPs. See FIG. 4. In tests with three different bacteria, pre-exposure of AgCNPs to irradiation increased the particles' antibacterial activity.

[0032] Testing of AgCNPs versus IrAgCNPs revealed the significant inhibition of *P. aeruginosa* in the IrAgCNP group. See FIG. 4A. Without irradiation, AgCNPs were unable to substantially eradicate *P. aeruginosa* and were five times less effective. See FIG. 4B. FIG. 4A (IrAgCNPS) and FIG. 4B (AgCNPs) show that the MIC for non-irradiated AgCNPs is 20 µg/mL and for IrAgCNPs is 100 µg/mL against *P. aeruginosa*. Thus, the non-irradiated AgCNPs are 5 times less effective here. Testing against *S. aureus* showed exposure to IrAgCNPs significantly reduced their growth and revealed a MIC of 200 µg. See FIG. 4C. However, AgCNPs reduced *S. aureus* growth less effectively and data revealed a MIC of 300 µg (i.e., a higher concentration is needed to eradicate the bacteria). FIG. 4C (IrAgCNPS) and FIG. 4D (AgCNPs) show that the MIC for non-irradiated AgCNPs is 300 µg/mL and for IrAgCNPs is 200 µg/mL against *S. aureus*. Testing against MRSA growth also showed the IrAgCNPs were more effective at inhibiting growth (See FIG. 4E) than the AgCNPs (See FIG. 4F). FIG. 4E (IrAgCNPS) and FIG. 4F (AgCNPs) show that the MIC for both non-irradiated AgCNPs and for IrAgCNPs is 300 µg/mL against MRSA, however IrAgCNPs are more effective at reducing overall MRSA growth starting at 60 µg/mL when compared with non-irradiated AgCNPs.

[0101] RNA sequencing analyses of MRSA confirm AgCNP-induced ROS dysregulation, and the withdrawal of essential intrinsic defense mechanisms for successful neutralization during oxidative attack. Additionally, data suggests an AgCNP-induced unique mechanism as displayed by an exhaustive, futile, but primary drive to survive *via* an anaerobic and ultimately high-metabolic cost fermentation process.

[0102] Example 4: Radiation Modification Effects.

[0103] The radiation-modified CNP and AgCNP surface increases the Ce^{3+} surface component (see FIG. 5A) thereby increasing hydrogen peroxide (H_2O_2) production. This, together with synergistic interaction with nitric oxide ($NO/NO_2^-/NO_3^-/NOx$) (see FIG. 5B), may have caused the increased antibacterial activity measured in AgCNPs over CNPs. These tests were carried out with bacteria and nanoparticles cultured within broth at 37^0C, and therefore with access to proteins and other biomolecules that will positively support bacterial growth. The antibacterial activity of both CNPs and AgCNPs are substantially improved when in the absence of these biofactors, and so these experiments represent a worst-case scenario. By adding broth, we are taking into consideration the potential presence of for example, human blood, or sputum on a hard surface (e.g., from patients in a hospital setting), that may help bacteria to grow despite the activity of the nanoparticles.

[0104] H_2O_2, and NO activity in the absence of bacteria was measured as follows. Levels of H_2O_2 activity were quantitated using a *Pierce™ Quantitative Peroxide Assay Kit* (Molecular Probes, Thermo Fisher Scientific™). The assay was performed at a sample:working reagent ratio of 1:2. The solution was gently mixed and incubated for 15-20 minutes at room temperature, and absorbance measured at 595 nm (Spectramax iD5 multimode microplate reader, Molecular Devices™). The concentration of peroxide was calculated by reference to its assay absorbance and using a standard curve. Control samples contained distilled water only. The generation of NO was quantified using the Griess Reagent Assay (Invitrogen™). 1 mL of each sample was incubated with 100 µL Griess reagent [1:1 ratio of 1% sulfanilamide in 10% orthophosphoric acid and 0.2% napthylethylenediamine in dH2O] for 15 minutes, in the dark, and at room temperature. Absorbance was measured at 548 nm relative to the reference sample (Spectramax iD3 multimode microplate reader, Molecular Devices™).

[0105] FIG. 5A shows hydrogen peroxide release from CNPs, and AgCNPs *versus* IrCNPs and IrAgCNPs. More harmful H_2O_2 is formed on the AgCNP *versus* CNP surface. When

AgCNPs and CNPs were irradiated, the levels of H_2O_2 formed significantly increased in a time-dependent manner. This likely contributes to the increased antibacterial activity measured in AgCNPs over CNPs, and with irradiated nanoparticles over non-irradiated particles.

[0106] FIG. 5B shows nitric oxide (NO) release and dissociates (($NO/NO_2^-/NO_3^-/NOx$) from CNPs, and AgCNPs *versus* IrCNPs and IrAgCNPs. No nitric oxide was generated by the water, confirming it to be a purely nanoparticle surface phenomenon. Exposure to radiation did not increase or decrease CNP or AgCNP NO levels. Significantly more NO was formed by AgCNPs *versus* CNPs (both with and without radiation), and this may also be at least partly responsible for the enhanced antibacterial activity measured with AgCNPs. This is because when H_2O_2 and NO combine, they form additional, and particularly harmful free radicals (e.g., singlet oxygen) which would also damage/kill bacteria.

[0107] Example 5: Example Production Method for AgCNPs.

[0108] The following is the steps for production of AgCNP1:

1. 4.35g of cerium nitrate hexa-hydrate (99.999% purity) is dissolved for every 100 mL of dH_2O stirred at 250 rpm; 425 mg of silver nitrate (99% purity) is dissolved in 25 mL of dH_2O under vortexing. Reactions:
   $Ce(NO_3)_3 \rightarrow Ce^{3+} + NO_3^-$; $AgNO_3 \rightarrow Ag^+ + NO_3^-$

2. In 1:4 ratio, aqueous silver is added to the cerium solution and allowed to mix under moderate (250 rpm) stirring for 5 min. Reactions: Mix: $(Ce^{3+} + NO_3^-)$, $(Ag^+ + NO_3^-) \rightarrow Ce^{3+} + Ag^+ + NO_3^-$

3. Stirring speed increased to 450 rpm and 25 mL of 0.4 M NaOH for every 125 ml of solution (step 2), is added drop-wise (via pipette); stirring maintained for additional 20 min. Reactions: $Ce^{3+} + Ag^+ + NO_3^- + NaOH \rightarrow Ce_x^{3+}(OH_2)_y|Ag^+ + Ag(OH) \rightarrow Ce(OH)_{4-x}|xAg^0 + Ag_2O + H_2O + O_2$

4. Solution is washed (centrifugation at 12,000 rpm (21, 000 rcf) for 10 min) 3x with dH_2O. Pellet is re-suspended in 87.5 mL of dH2O for every

125 ml of solution (step 2) and ultra-sonicated for 15 min at high intensity. Particle concentration determined by dry weight and dried material discarded after weight determination. Reactions: $Ce(OH)_{4-x}|(x/2)Ag^0 + Ag_2O + H_2O + O_2 \rightarrow CeO_{2-x/2}|xAg^0 + Ag_2O$

5. Stock particle suspension is ultra-sonicated, as above, and diluted to 4 mg/mL in 10% aq. NH_4OH. Solution is stirred at 250 rpm for 24 hrs. Upon digestion of silver and silver oxide secondary particle phases, solution changes color from deep/inky black to yellow/gold color. Reactions: $CeO_{2-x/2}|xAg^0 + Ag_2O + [NH_4OH \leftrightarrow NH_3 + OH^-] \rightarrow CeO_{2-x/2}|xAg^0 + [Ag(NH_3)_2]^+(H_2O)$

6. Solution is washed and re-suspended (in 75 mL of dH2O for every 125ml of solution (step 2)), and ultra-sonicated in similar manner to earlier steps. Particles are left to stand overnight. Unstable (sedimented) particle mass is separated from the suspended phase by aspiration. 8. Final concentration determined as dry mass (as step 4) and particles are ultra-sonicated prior to further use. Reactions: $CeO_{2-x/2}|xAg^0 + [Ag(NH_3)_2]^+(H_2O) \rightarrow CeO_{2-x/2}|xAg^0$

[0109] The following is an example of the steps for production of AgCNP2:

1. 228.27 mg of cerium nitrate hexa-hydrate (99.999% purity) is dissolved for every 100 mL dH_2O in a glass container. 523.55 µL of 0.2 M aq. $AgNO_3$ (99% purity) was added to the cerium solution and vortexed for 2 min. Reactions: $Ce(NO_3)_3 \rightarrow Ce^{3+} + NO_3^-$; $AgNO_3 \rightarrow Ag^+ + NO_3^-$

2. 4.19 mL of 3% hydrogen peroxide (stock) to every 100ml of dissolved cerium nitrate hexa-hydrate is added quickly to the solution from step 1 and immediately vortexed for 2 min at highest rotation speed. Reactions: $Ce^{3+} + Ag^+ + NO_3^- + H_2O_2 \rightarrow Ce^{3+}(OOH_2)|Ag^+ \rightarrow (Ce^{4+}(OH)_{4-(x+y)})(OOH)_x|yAg^0 + Ag^+$

3. Solution is stored in dark at room temperature with the bottle cap loose to allow for release of evolved gases. Solutions are left to age in these conditions for up to 3 weeks (monitoring solution color change from yellow to clear). Reactions: $(Ce^{4+}(OH)_{4-(x+y)})(OOH)_x|yAg^0 + Ag^+ \rightarrow CeO_{2-y/2}|yAg + H_2O + Ag^+$

4. Particles are dialyzed against 4.2 liters of dH2O over 2 days, (dialysis tubing – 3500Da) with the water changed every 12 hours and stored in the same conditions as for ageing. Reactions: $CeO_{2-y/2}|yAg + H_2O + Ag^+ \rightarrow CeO_{2-y/2}|yAg$

[0110] Briefly, a solution containing AgCNP-like, silver- modified nanoceria, and silver secondary phases are formed via an alkaline-forced hydrolysis reaction. The product materials are washed with dH_20 and subsequently treated with ammonium hydroxide. Ammonium hydroxide functions as an etchant as well as a phase transfer complex: mediating the solubilization/stabilization of dissolved silver ions in the aqueous phase. In particular, the reaction results in the formation of Tollen's reagent ($Ag[(NH_3)_2OH]aq$). The resulting single particle solution is then washed with dH_20 to remove excess base and counter/spectator ions.

[0111] AgCNP2 utilizes the stability of silver ions towards oxidation by hydrogen peroxide. Specifically, dissolution of cerium and silver nitrates followed by addition of hydrogen peroxide leads to the selective oxidation of cerium ions over silver and the evolution of metallic silver phases on the ceria surface. The unique synthesis conditions of these particles suggest a potentially disparate particle character. In certain embodiments the synthesis can be scaled to large or small processes.

[0112] A specific example for production of a small batch of IrAgCNP2 follows:

1. 109 mg of cerium nitrate hexa-hydrate (99.999% purity) were dissolved in 47.75 ml dH20 in a 50 ml square glass bottom bottle with removable lid.

2. 250 µl of 0.2 M aq. AgNQ3 (99% purity) was added to the cerium solution above with the solution vortexed for 2 minutes.

3. 2 ml of 3% hydrogen peroxide (stock) is added quickly to the above solution followed by immediate vortexing for 2 minutes at highest rotation

speed.

4. The solution is stored in dark condition at room temperature with the bottle (50 ml square bottom glass) cap loose to allow for release of evolved gases; solutions are left to age in these conditions for up to 3 weeks (monitoring solution color change from yellow to clear) to create 50 ml total volume of the solution.

5. Particles are then dialyzed against 2 liters of dH_20 over 2 days, with the water changed every 12 hours and stored in the same conditions as for ageing.

6. The particles then are irradiated at a dose of 10.5 Gy of irradiation, which was applied using a 160 kV tube voltage, 4 mA tube current, and a distance of 30 cm between the source and the surface (SC 500 smart controller, KIMTRON, USA).

[0113] Example 6: Comparative Testing.

[0114] Samples of CNP according to embodiments of the invention, which have been irradiated, were tested for comparison to non-irradiated prior art samples of CNP. The results are shown in Table 1, below.

[0115] Silver modified cerium oxide formulations (AgCNPs) were synthesized in two unique formulations (AgCNP1, AgCNP2) each utilizing different chemical reactions specific to aqueous silver. AgCNP1 was synthesized via a previously developed, two-step procedure. Briefly, a solution containing AgCNP-like, silver-modified nanoceria, and silver secondary phases are formed via an alkaline-forced hydrolysis reaction. The product materials are washed with dH_20 and subsequently treated with ammonium hydroxide. Ammonium hydroxide functions as an etchant as well as a phase transfer complex: mediating the solubilization/stabilization of dissolved silver ions in the aqueous phase. In particular, the reaction results in the formation of Tollen's reagent ($Ag[(NH_3)20H]aq$). The resulting single particle solution is then washed with dH_20 to remove excess base and counter/spectator ions. AgCN P2 utilizes the stability of silver ions towards oxidation by hydrogen peroxide.

Specifically, dissolution of cerium and silver nitrates followed by addition of hydrogen peroxide leads to the selective oxidation of cerium ions over silver and the evolution of metallic silver phases on the ceria surface. The unique synthesis conditions of these particles suggest a potentially disparate particle character.

[0116] Colloidal characteristics of the particles were evaluated for kinetic stability, surface potential, and hydrodynamic diameter. Dynamic light scattering (DLS) measurements of each sample are collected in Table 1 and relate a greater particle size (including specific spheres of hydration) for each formulation with AgCNP1 particles being -3x larger in diameter. Further, zeta potential measurements indicate a greater surface potential for AgCNP2 over AgCNP1, each with a positive polarity. These characterizations suggest the observation that AgCNP2 particles show greater kinetic stability over AgCNP1 (AgCNP1: moderate precipitation following particle ageing 1 week in room condition; AgCNP2: no observable sedimentation for greater than 5 months). AgCNP1 particles also present as turbid in solution at 1 mg/ml whereas AgCNP2 are completely translucent under similar conditions, suggesting greater Mie scattering related to larger particle size. Particles from each synthesis were observed to demonstrate unique fundamental and functional material character.

[0117] Table 1. Physicochemical Properties of Example CNP Formulations.

Property	CNP Tested	
	AgCNP1	AgCNP2
Ce3+:Ce4+ (%Ce3+)	25.8	53.7
[Ag]/[Ag+Ce]	16.9%	14.6%
SOD Activity (% Inhibition)	97.9%	99.2%
Hydrodynamic Diam. (nm)	42.2±4.6	16±5.1
Zeta Potential (m VJ)	22.4 ± 0.9	24.1 ± 1.3
Ecorr	465.386 mV	217.374 mV
Icorr	0.027 uA	0.013 uA
Beta*a*	681.7 mV	617.0 mV
Beta*c*	269.2 mV	21.8 mV

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[0118] All references listed below and throughout the specification are hereby incorporated by reference in their entirety.

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CLAIMS

What is claimed is:

1. An irradiated metal-associated cerium oxide nanoparticle (IrmCNP) comprising: cerium oxide doped with a metal, wherein the IrmCNP is irradiated so as to have a ratio of Ce3+:Ce4+ on the surface of the nanoparticle of 60% or greater.
2. The cerium nanoparticle of claim 1 wherein the metal is selected from the group consisting of silver, gold, ruthenium, vanadanium, copper, titanium, nickel, platinum, titanium, tin and iron, the metal optionally being in ion form.
3. The IrmCNP of claim 2, wherein the metal is silver.
4. The IrmCNP of claim 3, wherein the silver is in an amount of less than 10% by weight relative to the cerium.
5. The IrmCNP of claim 3, which is doped with fluorine.
6. The IrmCNP of claim 1 which is less than 100 nm, less than 50 nm, less than 25 nm, less than 15 nm or less than 10 nm in size.
7. The IrmCNP of claim 4 which is less than 100 nm, less than 50 nm, less than 25 nm, less than 15 nm or less than 10 nm in size.
8. The IrmCNP of claim 1, wherein the nanoparticle releases H_2O_2 at a rate of at least about 1.5 to about 2 times higher than the same mCNP without irradiation.
9. A method of making an IrmCNP comprising:
    (a) dissolving cerium and silver nitrate salts;
    (b) oxidizing the dissolved cerium and silver salts by admixture with peroxide;
    (c) precipitating nanoparticles by adding ammonium hydroxide to the oxidized cerium and silver salts;
    (d) washing and resuspending the precipitated nanoparticles in water;
    (e) adding hydrogen peroxide to the resuspended nanoparticles;
    (f) washing the nanoparticles from (e) to remove ionized silver; and
    (e) irradiating the precipitated nanoparticles at a dose of 10.5 Gy.
10. A cerium nanoparticle made according to the method of claim 9.
11. A IrmCNP composition comprising the IrmCNP of claim 1 and a solvent or solvent mixture excipient.
12. The IrmCNP composition comprising the cerium nanoparticle of claim 10 and a solvent or solvent mixture excipient.
13. The IrmCNP composition of claim 11, wherein the solvent or solvent mixture excipient

is selected from the group consisting of water, chloroform, methylene chloride, acetone, methyl ethyl ketone, cyclohexane, ethyl acetate, diethyl ether, lower alcohols, lower dials, THF, DMSO, DMF, and any combination thereof.

14. The IrmCNP composition of claim 12, wherein the solvent or solvent mixture excipient is selected from the group consisting of water, chloroform, methylene chloride, acetone, methyl ethyl ketone, cyclohexane, ethyl acetate, diethyl ether, lower alcohols, lower dials, THF, DMSO, DMF, and any combination thereof.

15. The IrmCNP composition of claim 11, further comprising one or more of a drying agent, an organic acid, one or more surfactants, a polymer binder, and water.

16. The IrmCNP composition of claim 12, further comprising one or more of a drying agent, an organic acid, one or more surfactants, a polymer binder, and water.

17. A method of disinfecting a surface, comprising contacting the surface with the IrmCNP composition of any of claims 11-16.

18. An article comprising the nanoparticle of any of claims 1-8 and 10 or the composition of any of claims 11-16 disposed on a surface thereof.