Potent Inactivation of Human Respiratory Viruses Including SARS-CoV-2 by a Photoactivated Self-Cleaning Regenerative Antiviral Coating

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ABSTRACT: The COVID-19 pandemic marks an inflection point in the perception and treatment of human health. Substantial resources have been reallocated to address the direct medical effects of COVID-19 and to curtail the spread of the virus. Thereby, shortcomings of traditional disinfectants, especially their requirement for regular reapplication and the related complications (e.g., dedicated personnel and short-term activity), have become issues at the forefront of public health concerns. This issue became especially pressing when infection-mitigating supplies dwindled early in the progression of the pandemic. In consideration of the constant threat posed by emerging novel viruses, we report a platform technology for persistent surface disinfection to combat virus transmission through nanomaterial-mediated, localized UV radiation emission. In this work, two formulations of Y2SiO5-based visible-to-UV upconversion nanomaterials were developed using a facile sol–gel-based synthesis. Our formulations have shown substantial antiviral activities (4 × 10⁴ to 0 TCID₅₀ units in 30 min) toward an enveloped, circulating human coronavirus strain (OC43) under simple white light exposure as an analogue to natural light or common indoor lighting. Additionally, we have shown that our two formulations greatly reduce OC43 RNA recovery from surfaces. Antiviral activities were further demonstrated toward a panel of structurally diverse viruses including enveloped viruses, SARS-CoV-2, vaccinia virus, vesicular stomatitis virus, parainfluenza virus, and Zika virus, as well as nonenveloped viruses, rhinovirus, and calicivirus, as evidence of the technology’s broad antiviral activity. Remarkably, one formulation completely inactivated 10⁵ infectious units of SARS-CoV-2 in only 45 min. The detailed technology has implications for the design of more potent, long-lived disinfectants and modified/surface-treated personal protective equipment targeting a wide range of viruses.

KEYWORDS: COVID-19, SARS-CoV-2, virucide, self-cleaning surfaces, self-sanitizing coating, upconversion nanomaterials, photoactivated regenerative virucide

1. INTRODUCTION

Viral infections are a major public health concern, with seasonal (e.g., influenza virus), ubiquitous (e.g., norovirus), and pandemic (e.g., SARS-CoV-2) viral pathogens imposing a sizable burden on the economy and general society. Non-influenza-related infections account for ~$40 billion annually in medical costs (United States), with total costs comparable to those from chronic conditions like hypertension and congestive heart failure.¹ Viruses can be difficult to eliminate or inactivate due to their small size, inherent stability, and high infectivity per particle.² Many respiratory virus particles can remain infectious on surfaces (e.g., hospitals, rest homes, and airports) for long periods of time, including rhinoviruses,³ coronaviruses, and influenza viruses.⁴,⁵ For example, human coronavirus (HCoV) 229E infectivity is still detectable after 3–48 h on surfaces of varying character (aluminum, sterile latex surgical gloves, sterile sponges, and plastic), with chemical disinfectants such as providine only able to reduce the viral infectious titer up to 50%.⁶ SARS-CoV-1 was found to remain infective on a disposable medical gown for up to 2 days, on cloth for 24 h, and on stainless steel for 48 h. SARS-CoV-2 has been found to remain infective on surgical masks and steel for >7 and >8 days, respectively.⁷,⁸ Influenza A-virus can exist on a stainless-steel surface for 2 weeks.⁹ The surface transmission of viruses is particularly problematic for very stable and highly infectious virus species, such as the norovirus, an enteric virus, which is estimated to affect 1 in every 15 US residents each year as the leading cause of food-related illnesses.¹⁰ For noroviruses, the stability of infectious particles can be greatly influenced by the nature of the contaminated surface, as well as residual food and organic debris.¹¹ Thus, there is intense

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interest in studying the effects of material characters for high-touch surfaces on disinfectant potency and broad-spectrum efficacy, especially for the development of long-lasting disinfectants.

The devastating effects (>528 million confirmed cases, with more than 6 million deaths worldwide) from the spread of SARS-CoV-2 and the limited efficacy of counter/containment measures have highlighted the need for substantial improvements in antiviral technologies [WHO Coronavirus (COVID-19) Dashboard—WHO Coronavirus (COVID-19) Dashboard with Vaccination Data-covid19.who.int-retrieved 6-3-2022]. Furthermore, the emergence of new variants of the virus (gamma, delta, Omicron (BA.1), and BA.2.12.1 sublineages) with even greater transmission properties are a stark reminder to the scientific community of the growing need for better methods to limit the spread of new viral strains. In particular, preventing surface-to-surface transmission will be essential, given the pronounced ability for SARS-CoV-2 to remain active on various surfaces. One of the studies conducted on a COVID ward in a hospital (Milan, Italy) reported that different surfaces of the ward were COVID-positive. More clarity on this issue came with a study on the activity of the virus on dry surfaces. Specifically, the virus was observed to remain active for 48 h (h) on plastics, 24 h on stainless steel, 8 h on copper, and 24 h on cardboard. These reports, in aggregate, point to the specific challenge of surface-to-surface spread in infection-prone areas and suggest the necessity for self-cleaning surfaces and personal protective equipment (PPE).

Broadly, there are two main categories of viricides: the more commonly found approach being chemical agent-based virucide and the other based on physicochemical methods. The most commonly used chemical viricidal disinfectant is ethyl alcohol which can cause rapid denaturation of viral proteins in the presence of water. Another such common antiviral chemical agent is chlorhexidine, which is suggested to disrupt the envelope of the virion. However, its antiviral activity is limited to enveloped viruses. The prevalence of chemical viricudes is evident in the variety of agents in use, such as benzalkonium chloride, hand soap, cranberry extracts, chloroxylene, and chlorhexidine. Most chemical agent-based disinfectants are not regenerative or self-cleaning in nature due to their mode of virucidal action. Therefore, these agents exhibit relatively short times of efficacy (nonpersistence of disinfection over time). Given the detailed limitations of these common approaches, new research into alternative viricidal agents saw an uptick in interest and the number of scientific publications during the early months of the COVID-19 pandemic. Studies on the structure and stability of SARS-CoV-2 spurred developments of more robust, specific modes of disinfection, in particular, as a response/consideration of the virus’s dense glycan shield at the spike protein surface.

To address such virus-specific challenges, recent reports have suggested nanoparticle-mediated disinfection, with various compositions, such as shellac/copper nanoparticles irradiated with solar light, silver/silica nano-particles on PPE masks, and silver-modified ceria nanoparticles as a liquid suspension to deactivate the SARS-CoV-2 virus. Photoactivated graphene oxide materials have been also used for antimicrobial materials.

The second viricidal approach is also used extensively and utilizes physicochemical approaches to disinfection. Among these approaches, ultraviolet (UV)-radiation-based disinfection has shown the greatest efficacy and is supported by a significant volume of reported studies. Largely, UV light exposure has been shown to induce point mutations in viral genetic material; inhibiting viral replication in an infected cell. Nevertheless, this approach has its own limitations; in particular, it generally requires external emission sources and affects a focused, limited area of action.

Despite some progress in this area of viricides, products with regenerative and self-cleaning characteristics remain elusive. In consideration of these criteria, the present study combines the advantages of a localized UV radiation emission with a broad-spectrum, white light-absorbing agent to produce physicochemical disinfection without necessitating a dedicated power source or bulky instrumentation/apparatus.

Upconversion nanoparticles (UCNPs) designed in the current study upconvert absorbed white light available as sunlight and common, indoor artificial lights [light-emitting diodes (LEDs), incandescent or fluorescent] to localized UV radiative emission. UV emission is persistent: allowing continuous disinfection under common light conditions. Typical inorganic phosphors have been produced from stable inorganic matrix materials with active rare-earth ions at dopant concentrations, mediating upconversion via narrow band-gap levels between higher-energy electron orbitals. Typical matrix-suited compositions are easily doped with heteroatoms, leading to minimal nonradiative relaxations, possessing high optical transparency, and demonstrating good chemical/thermal stability. In the presented study, yttrium silicates (Y2SiO5; matrix materials) have been doped with Pr5+, Gd3+, and Li+. UCNP materials were well characterized using X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), transmission electron microscopy (TEM), and fluorescence spectroscopy techniques with material compositions and processing procedures subsequently optimized for upconversion efficiency to UV wavelength emissions. UCNP materials were then coated on quartz slides and tested for antiviral properties against human coronavirus OC43, a widely circulating seasonal human respiratory virus. We similarly show that UCNPs are highly effective at inactivation of a broad range of viruses: (1) SARS-CoV-2; (2) rhinovirus, a very stable pathogen responsible for a large burden of upper respiratory infections; (3) parainfluenza virus, responsible for major lower respiratory infections predominantly in young children; (4) vesicular stomatitis viruses, a prototypic animal pathogen; (5) calicivirus, a surrogate for human norovirus; and (6) vaccinia virus, a large and complex DNA virus related to the human smallpox virus. The objective of the study was to establish UCNPs as potential regenerative antiviral agents and serve as proof of concept for future studies regarding UCNP antiviral coatings on various surfaces.

2. RESULTS AND DISCUSSION

Upconversion of light involves absorption (excitation) of higher-wavelength (low energy) light and emission of lower wavelength (higher-energy light). In most reported mechanisms, upconversion usually involves multiple photon excitations. In the case of our materials, visible-to-UV upconversion is accomplished via two-photon excitations. As noted earlier, upconversion nanomaterials usually consist of two primary functional components: host lattice/matrix material and dopant ions. In the present study, we have used Y2SiO5 as the host lattice and Pr, Gd, and Li as dopants. Yttrium silicates have been a popular choice for phosphor
Figure 1. A,B) shows the XRD pattern for $Y_2SiO_5$ (1% Pr and 1% Gd) [UCNP2] and $Y_2SiO_5$ (1.2% Pr, 1.2% Gd, and 7.2% Li) [UCNP1]. 1200 °C-calcined phase and 1400 °C-calcined phase both are shown for each sample (stacked). Low-temperature X1 (monoclinic, ICDD 01-070-5613) and high-temperature X2 (monoclinic, ICDD 01-084-4282) phases were observed in UCNP2 and UCNP1 samples, in 1200 °C-calcined samples, phase composition exists as a mixture of X1 and X2 phases. For 1400 °C-calcined samples, the X2 phase is in majority in both samples (peaks at 27.33, 28.5, and 29.2 2θ). The X2 phase is more prominent in the UCNP1 sample, independent of temperature. The coordination number of Y atoms is 7,9 in X1 and 6,7 in X2 phases. 1400 °C-calcined samples are used for further characterization and application. X-ray photoelectron spectra of $Y_2SiO_5$ (1% Pr and 1% Gd) (UCNP2) powders are shown in C (survey), D(Y3d), and E (Pr3d). Similarly, spectra of $Y_2SiO_5$ (1.2% Pr, 1.2% Gd, and 10% Li) (UCNP1) powder are shown in C (survey), F (Y3d), and G (Pr3d). The X2 phase is more prominent in the UCNP1 sample.
material host lattices due to their physicochemical stability and ease of rare-earth element (Ln\(^{+}\)) ion doping and solid solubility. Ln\(^{+}\) ions in the lattice are the active species in the anti-Stokes absorption/emission process. Partially filled 4f orbitals are a requirement in the active ions, limiting the choice of candidate lanthanide species. The energy and separation among intermediate electronic levels determine excitation and emission wavelengths for these materials. Upconversion mechanisms can be broadly classified into five different categories. (1) ESA: excited-state absorption; (2) ETU: energy transfer mechanism; (3) CET: cooperative energy transfer; (4) EMU: energy migration mediation; and (5) PA: photon avalanche. We are interested in ESA and ETU mechanisms for the presented work, as explained in detail in the subsequent sections.

For visible-to-UV-light upconversion, \(\text{Y}_2\text{SiO}_5\) (Pr doping) has been reported to have emission of around 260 nm wavelength UV radiation with blue-light excitation.\(^{2-16}\) There have also been reports of excitation by various visible-light spectra (violet, cyan, green, and yellow) which still demonstrate emission in the UV range.\(^{16,34}\) Visible-to-UV-upconverting nanoparticles (UCNPs) for this work were fabricated using a facile, scalable sol–gel-based method, yielding particles of irregular shape and morphologies.

### 2.1. Basic Properties of UCNPs

#### 2.1.1. Particle Formulation and Phase Analysis

XRD was performed to probe phase formation under synthesis conditions, as the upconversion process is highly sensitive to phase composition and structure: making these parameters vital to material performance/activity. XRD patterns for \(\text{Y}_2\text{SiO}_5\) (1% Pr and 1% Gd) (UCNP2) and \(\text{Y}_2\text{SiO}_5\) (1.2% Pr, 1.2% Gd, and 7.2% Li) (UCNP1) are shown in Figure 1A,B, respectively. XRD analysis was also performed on UCNP1 and UCNP2 samples following calcination at 1200 or 1400 °C to understand the effects of dopant content and temperature on the crystal structure.

Two phases of yttrium silicate (\(\text{Y}_2\text{SiO}_5\)), \(X_1\) (Monoclinic, ICDD: 01-070-5613) and \(X_2\) (Monoclinic, ICDD: 01-084-4282), are present in both samples. The \(\text{Y}_2\text{SiO}_5\) lower-temperature phase \(X_1\) has a monoclinic crystal structure with the \(P2_1/c\) space group. \(Y^{3+}\) occupies two different sites in the lattice in 7- and 9-coordinated sites in the monoclinic crystal. The higher temperature phase \(X_2\) also has a monoclinic crystal with an \(I2/a\) \((C2/c)\) space group, \(Y^{3+}\) ions occupy 6- and 7-coordinated sites in the crystal. Different phase-transformation temperatures have been reported in the literature for the \(X_1\) to \(X_2\) transition. The \(X_2\) phase has been reported to have better upconversion characteristics.\(^{34,35}\)

Therefore, in the present study, we have investigated the effects of various calcination temperatures on \(X_1/X_2\) phase compositions and on the conversion of \(X_1\) to \(X_2\) under thermal treatment. The transformation temperature was reported to be 850 °C by Nowok et al.,\(^{37}\) 1190 °C by Wang et al.,\(^{38}\) 1150 °C by Cannas et al.,\(^{39}\) 1250 °C by Cates et al.,\(^{42}\) and 1230 °C by Muresan et al.\(^{40}\) As evidenced by the presence of peaks corresponding to the \(X_1\) and \(X_2\) phase, 1200 °C-calcined samples of both UCNP1 and UCNP2 have mixed-phase constituents with a significant presence of the \(X_1\) phase. However, samples calcined up to 1400 °C show the \(X_2\) phase in the majority, in line with earlier reported works.\(^{32,40}\) Clear differences in XRD patterns exist between UCNP1 and UCNP2 samples heated to 1400 °C. Specifically, the samples show disparate fractions of the \(X_1\) and \(X_2\) phases, as identifiable in the greater relative intensities of \(X_2\) phase-related peaks (e.g., \(27.33, 28.5,\) and \(29.2 \pm 20\)) in the UCNP1 samples. Based on peak intensity ratios, a qualitative comparison of the relative \(X_1\) to \(X_2\) phase presence can be made, suggesting a more pronounced UCNP1 fraction for the samples calcined at 1400 °C. As noted above, lithium-doped UCNP1 shows a greater fraction of the \(X_2\) phase as compared to UCNP2 at comparable Pr and Gd amounts. The presence of added lithium in the UCNP1 formulation is suggested to account for this distinction, with lithium doping reported to aid in the polymorphic transformation (\(X_1\) to \(X_2\)). Furthermore, lithium doping, such as that employed in the present study, has been shown to produce a flux effect: preventing cross-relaxation by stabilizing Pr\(^{3+}\). These characters together thereby improve upconversion activity.\(^{34,46}\) While the relative fraction of \(X_2\) is low, there is a significant amount given the comparatively low (1200 °C for 24 h) calcination temperature. Furthermore, other studies have reported as much as 82 ± 2% \(X_2\) phase formation for a similar sample calcined at 1200 °C.\(^{37,39}\) The limited \(X_1 \rightarrow X_2\) transformation observed in the current study has been noted in other studies, for example, by Sun et al., as incomplete polymorph transformation.\(^{41}\) While incomplete polymorph transformation is common, it may be more pronounced in the current study due to the sol–gel-based nanomaterial synthesis approach, as opposed to bulk sintered products. Size dependency on phase-transformation kinetics has been explored before and reported to be significant differences.\(^{42}\) We have also performed differential scanning calorimetry and thermogravimetric analysis of UCNP1 and UCNP2 formulations prior to calcination, and the results are shown in Figure S2 (Supporting Information).

From these studies, we have observed that the initial formation of the \(X_1\) phase may occur at a lower temperature for the lithium-doped sample [UCNP1].

#### 2.1.2. Surface Chemistry Analysis

XPS was done to study the chemical states of dopants in each sample and determine any relations to upconversion performance. Figure 1C–G shows XPS spectra of \(Y_3d, \text{Pr} \_3d,\) and survey spectra for UCNP2 and UCNP1 samples. Survey spectra (Figure 1C)
confirm the presence of yttrium, silicon, and oxygen as majority components with trace praseodymium and gadolinium. Scans over the Si2p binding energy region show peaks attributed to Si−O interactions. Scans related to Pr3d show typical Pr3+ doublet peaks in both samples. In the Y3d scan shown in Figure 1D, for the UCNP2 sample, the deconvoluted peaks of 3d5/2 (UCNP2: 157.8 and 159 eV) and 3d3/2 (UCNP2: 159.9 and 161.1 eV) represent two different types of Y atoms: ascribed to yttrium sites from X1 (P21/c, primitive) and X2 (I2/a, end-entered) phases. Our observation is in line with earlier reported XPS analysis of yttrium silicates.

Variation in signal intensity (Figure 1D,F) corroborates trends in X1/X2 content for each formulation, observed in XRD measurements (Figure 1A,B) discussed above. Lithium content in the sample is limited (the XPS survey spectrum has been shown in Figure 1C). However, this character is expected due to our high calcination temperature (1400 °C) and the low boiling points of lithium compounds (lithium nitrate was added). Gd was observed in the samples at trace levels.

2.1.3. Particle Morphologies. The size and morphological characteristics of UCNPs from each formulation were analyzed by high-resolution transmission electron microscopy (HRTEM), as shown in Figure 1H−N. HRTEM images of UCNP2 formulation are shown in H and I. As expected from a sol−gel-based synthesis route before thermal treatments at higher temperatures, the majority of nanoparticles have irregular shapes with sizes around 5 nm (after sieving) (size estimation using ImageJ software also done, shown in the Supporting Information: PL spectrum of UCNPs and size distribution estimation using TEM image). As-prepared nanoparticles were subjected to sieving (see the Materials and Methods section for more details) to separate coarser particles. The Figure 1I HRTEM image shows an interplanar spacing estimated to be ∼0.299 nm (determined using digital micrograph software and inverse fast Fourier transform). A single nanoparticle was isolated in HRTEM and subjected to electron diffraction; a related and representative diffraction pattern is shown in Figure 1J for which the selected-area diffraction pattern crystallographic axis was determined to be [01̅1]. Observations and analysis are in agreement with earlier published work.38 HRTEM analysis was collected for UCNP1 samples and is shown in Figure 1K−N. The Figure 1L HRTEM image shows interplanar spacing, which was estimated to be 0.205 nm. Particles from this method are similar in size and shape to those from UCNP2. Size distribution has been estimated using TEM image and ImageJ software; the mean size is ∼4.239 ± 1.176 nm. Analysis from this measurement (PL spectrum of UCNPs and size distribution estimation using the TEM image) is shown in Figure S1B,C. Figure 1N shows an image of the nanoparticles at lower magnification to evidence the limited particle size dispersion.

2.2. Visible-to-UV-Light Upconversion. Y2SiO5 (dopant: Pr, Gd, and Li) nanomaterials are used in this work for the upconversion process, therein, by two-photon excitation of visible light followed by emission in the UV range. Two mechanisms govern the upconversion process; specifically, ESA and ETU (a schematic diagram of the ESA and ETU processes is presented in Figure 2). In our work utilizing...
Y₂SiO₅ (Pr, Gd, and Li), ESA is common among all samples [photoluminescence (PL) graph shown in Figure 2]. PL measurements for other compositions of dopants shown in Figure S1. Gd codoping introduces another ETU mechanism to the system. Pr³⁺ is the key dopant responsible for emission in the UVC region. In the ESA process, an electron is excited from $^3H_4 \rightarrow ^3P_j \rightarrow 4f5d$ at a Pr³⁺ site (shown in Figure 2B,C); subsequent relaxation through the Pr³⁺ ion gives photon emission around 260 nm. For Gd codoping with Pr, ETU can lead to another emission process. Specifically, electron excitation from $4f5d \rightarrow ^6I_j$, then subsequent relaxation to $^6P_j \rightarrow ^8S_7/2$, results in emission around 316 nm (UVB region). However, the ETU process can happen in only Pr-doped samples, as shown in Figure 2B. Li codoping improves upconversion efficiency by preventing cross-relaxation. In particular, the presence of lithium in the material structure limits Pr³⁺ clustering: causing individual ions to disperse throughout the crystal. 

The PL spectra of UCNP1 and UCNP2 (shown in Figure 2) show a slight difference in their emission characteristics when excited with the same excitation source. Both formulations have emissions within the UVC (100–280 nm) and UVB (280–320 nm) ranges. However, the emission peak for UCNP1 within the UVC region is blue-shifted (shifted toward a lower wavelength) compared to UCNP2. Additionally, the UCNP2 formulation was observed to emit over a broader range: with significant UVB range emission observed. Given the synthesis procedure was identical between the formulations, changes in the respective emission characters are ascribed to differences in material composition and, specifically, to the effects of lithium incorporation into UCNP1. Similar trends in blue shifting of UVC absorption intensity were observed in other formulations with lithium doping for Y₂SiO₅ (Pr) and Y₂SiO₅ (Pr and Li) compositions, as shown in Figure S1 (Supporting Information), which is in line with earlier published work. Given the distinct emission characters of each particle formulation, we looked to investigate their specific antiviral activities and any difference in activity or efficacy between each formulation. Antiviral testing results are discussed in later sections.

### 2.3. Antiviral Properties of UV Radiation

UV radiation induces biological modifications and chemical transformations in microorganisms through wavelength-specific photophysical and photochemical processes, which affect their survival and activity. These effects occur through both direct (physical) and indirect (through reaction with a radiation-induced chemical species) pathways. While direct effects often lead to substantial damage, indirect pathways affect a wider range of biological components and are more prevalent. Often, these effects occur through reactive oxygen species: potentially producing oxidative damage to membrane (coatings) lipids and proteins as well as to genetic materials. Absorption of UV radiation is wavelength-dependent, with consequent responses to UV radiation also being wavelength-dependent. The shorter-wavelength (higher energy) UVC region has been shown to interact strongly with RNA/DNA: inducing electron excitation of unsaturated chemical species, with aromatic purines and pyrimidines being susceptible to dimerization and cross-linking reactions. Furthermore, cross-linking has been shown to occur between photoactivated nucleobases and structural components such as viral capsid structures. In a similar manner to nucleobase inactivation, UVC is readily absorbed by aromatic amino acids (e.g., phenylalanine and tyrosine). For this reason, UVC has been referred to as
Figure 4. OC43 coronavirus inactivation studies. (A) TEM image of OC43 virions (deactivated), with spike protein visible on the virion surface. (B) Approximately $1 \times 10^5$ TCID$_{50}$ units of OC43 were applied to slides and exposed to UV light for the times indicated. At each time point, slides were processed, and the remaining infectivity was determined by the TCID$_{50}$ assay. Slide measurements taken prior to UV light exposure are indicated as time 0. Values are the mean of three independent sample replicates. A UV dose curve was then estimated using a radiometer to quantify effective UV doses to the viruses per source setting (more details in Section 4). UV dosage from a rated light source, and corresponding viral deactivation, were also implemented to estimate UV emission from UCNPs. (C–E) Approximately $1 \times 10^5$ TCID$_{50}$ units of OC43 were applied to untreated (Unt) or UCNP-coated slides. Slides were then covered (Cov) or exposed (Exp) to LED light for 1 h (C); 10, 20, or 40 min (D); or for 30 min (E). As shown in D, viruses were not deactivated upon 10 or 20 min exposure, but 40 min of exposure led to complete deactivation. We followed this experiment with a 30 min exposure and observed that all the viruses were deactivated in 30 min too, as shown in (C). At the indicated times, slides were processed, and the remaining infectivity was determined by the TCID$_{50}$ assay. Slides taken prior to light exposure are indicated as time 0. OC43 RNA recovery was reduced from UCNP1- and UCNP2-coated slides when exposed to light-exposed. (F) Schematic representation of the RNA recovery experiment. (G,C) $\sim 1 \times 10^5$ TCID$_{50}$ units of OC43 were applied to untreated (Unt) or UCNP-coated slides in triplicate. Slides were then covered (Cov) or exposed (Exp) to LED light for 1 h and pooled in media. Media were then processed for RNA extraction followed by RT-PCR amplification of the Spike gene. PCR products were visualized on an agarose gel (G), and band intensities from this representative experiment (G) were measured and normalized as a percentage of corresponding covered controls (H). Values are the mean of three independent sample replicates with error bars representing the standard deviation. Un represents undetectable virus infectivity. *indicates $p$-value < 0.05 and ** indicates $p$-value < 0.01 comparing untreated light-exposed slides to the UCNP-coated light-exposed slides. UCNPs were coated to approx. 2–3 μm thickness on the glass slides (more details in the Materials and Methods section).
germicidal UV. Longer-wavelength UVB radiation is often associated with lipid peroxidation, protein carbonylation, and metabolic activity loss.\textsuperscript{48} UVB also incurs an extent of DNA damage but is less potent (with respect to viral inaction), in comparison to UVC. It should be noted that RNAs are known to be more susceptible to UVC than UVB.

In our current study, we initially looked to identify UCNP efficacy toward human coronaviruses. Therefore, we have arranged the presented antiviral studies by first considering human coronavirus OC43 and SARS-CoV2 (discussed in a later section). Our studies were then extended to probe a wide variety of structurally unique virus types to determine any trends in antiviral action which could be generalized, based on results from the complete study (discussed in a later section). Below, we consider a human coronavirus as an example/model virus and note-specific, relevant UV photoreactions, potentially leading to the observed inactivation by our materials. Furthermore, the wavelength-dependent sensitivities of each process are considered with respect to the emission characters of each UCNP formulation.

A schematic diagram showing a generic human coronavirus structure with relevant UV radiation damage mechanisms overlain is shown in Figure 3. Human coronaviruses possess a single-stranded RNA genome. As noted earlier, RNA is known to strongly absorb UVC radiation: producing dimers of cytosine and uracil pairings (as well as hydrated radicals). Furthermore, single-stranded RNA/DNA are noted to be more sensitive to photoinactivation than double-stranded structures (possibly due to conferred stability from $\pi$-stacking).\textsuperscript{49}

Proteins, such as those within the viral envelope, also show degradation from UV irradiation through disruption of secondary structural conformations due to photohydrolysis reactions.\textsuperscript{50,51} Additionally, protein amino acid composition can be a determinant in predicting UV degradation. Primary structures that include aromatic amino acids such as tyrosine or tryptophan absorb UVC region radiation, leading to photoexcitation and radical induction following reactions with chemical species such as those containing disulfide bonds (effecting reduction and radical propagation).\textsuperscript{52} Disassociation of such disulfide bonds can create loss-of-function deformations in proteins. Finally, lipids often undergo oxidation under UV light; however, specific UV-C range irradiation does not show this reaction.\textsuperscript{53}

Disassociation of such disulfide bonds can create loss-of-function deformations in proteins. Finally, lipids often undergo oxidation under UV light; however, specific UV-C range irradiation does not show this reaction.\textsuperscript{53} Free radicals are the major photoproduct from UV radiation due to the lipid losing a hydrogen atom. This is especially true among unsaturated fatty acids which allow for easier removal of hydrogen atoms and subsequent free radical formation.\textsuperscript{54} The singlet oxygens created from irradiation on lipids can cause subsequent oxidative damage to other biomolecules at more distant chemical sites.

In a recent study, a quantitative evaluation of viral inactivation using UV radiation was done on SARS-CoV-2.\textsuperscript{55} A quantitative assessment of antiviral activity using 265, 280, and 300 nm UV radiation has shown the dependency of the UV radiation source and dosage (exposure time). 265 nm light needed the least exposure time (dosage) to deactivate the virus titer, and it also happens to have the highest energy (the lower the wavelength of the source, the higher the energy).\textsuperscript{55}

2.4. Inactivation of Human Coronavirus OC43 by Direct UV Light. UV light effectively inactivates viruses, with specific virus structures suggested to confer varying susceptibilities to UV-mediated inactivation.\textsuperscript{56} Critical UV-light dosing was optimized for the inactivation of human coronavirus OC43, an enveloped single-stranded positive-sense RNA
(+ssRNA) virus with a genome size of about 30 kilobases (kb). The OC43 virion size ranges from 120 to 160 nm in diameter, as shown in the TEM image (Figure 4A). To generate a UV-light dose curve required for OC43 virus inactivation, approximately $10^5$ infectious TCID\(_{50}\) units of OC43 virus were delivered to glass slides, and infectivity recovered at time zero was determined. Slides were then exposed to 254 nm UV light for varying durations, followed by a medium wash, and processed for infectious virus recovery. UV light exposure reduced viral titers from $10^5$ TCID\(_{50}\) units to undetectable levels by 30 s of exposure. About a 10 mJ/cm\(^2\) UV light dose is required for complete OC43 virus inactivation (Figure 4B) (light source, a radiometer, and other details are provided in the Materials and Methods section). More details are provided in Figure S4 (Supporting Information). UV light dose required to inactivate coronaviruses. This experiment with the OC43 virus also highlights the dose/exposure time dependency of viral inactivation, which is in line with the observation reported with SARS-CoV-2.\(^5\)

2.5. UV-Light-Generating UCNP-Coated Slides Inactivated Human Respiratory Coronavirus OC43 and SARS-CoV-2. A schematic representation of the experimental setup for testing the ability of UCNP-coated slides to inactivate viruses is shown in Figure 5. Uncoated or UCNP-coated slides were prepared, and approximately $1 \times 10^5$ TCID\(_{50}\) or plaque forming unit (PFU) of the respective virus was delivered to each slide. Control slides were covered with tin foil, and exposed samples were illuminated directly with an LED light. At the indicated incubation times, slides were washed in media. Samples were then analyzed for infectivity according to respective virus quantification assays. To determine the extent to which UCNP-coated slides could inactivate human coronavirus OC43, $\sim 10^5$ infectious TCID\(_{50}\) units of OC43 virus were applied to untreated or UCNP1- or UCNP2-coated glass slides. The $10^5$ TCID\(_{50}\) input virus was determined as time zero infectivity. Slides were then covered or light-exposed for 1 h, followed by a media wash, and samples were processed for the remaining infectious virus. Untreated exposed samples retained about $10^5$ TCID\(_{50}\) units of infectious OC43 (Figure 4C). By contrast, both the light-exposed UCNP1- and UCNP2-coated slides reduced OC43 viral infectivity from $\sim 10^5$ TCID\(_{50}\) units to undetectable levels (un).

Since a 1 hour light exposure was sufficient for UCNP-coated slides to inactivate OC43 infectivity completely, we next determined the optimal exposure time required for OC43 viral inactivation. Approximately $10^5$ infectious TCID\(_{50}\) units of OC43 were delivered to untreated or UCNP1- or UCNP2-coated glass slides. Slides were then covered or light-exposed for 10, 20, or 40 min, followed by a medium wash and processed for infectious OC43 (Figure 4D). Approximately 40 min of light exposure was required to completely inactivate OC43 infectivity by slides coated with UCNP1 (purple line) and UCNP2 (black line). In a shorter time course, 30 min of light exposure was sufficient for UCNP-coated slides to inactivate OC43 (Figure 4E) fully, and this was not seen in control samples of UCNP-coated slides that were left unexposed to light. Exposure time-dependent antiviral properties are in line with our earlier observation with the 254 nm UV light source. Longer exposure to white light will lead to a higher dosage of upconverted UV radiation and improve antiviral activities. It can be claimed that (1) UV light-mediated virus inactivation is time-dependent (Figure 4B), (2) UCNP coatings upconvert white light to UV light (Figure 2), and (3) white light-exposed UCNP coatings inactivate viruses in a time-dependent manner.

To confirm that virus particles were removed from the slides during the washing step, levels of viral RNA recovered off slides after inactivation were determined by RT-PCR. Approximately $10^5$ infectious TCID\(_{50}\) units of OC43 were administered to untreated or UCNP1- or UCNP2-coated glass slides and were left covered or exposed to LED light for 1 h. Following a medium wash, samples were then processed by RNA extraction, cDNA synthesis, PCR amplification of the spike gene, and gel visualization (Figure 4F). Band intensity quantification demonstrated spike RNA recovery of the untreated exposed samples was about 100% of the untreated covered control slides, whereas both UCNP1- and UCNP2-coated slides reduced RNA recovery to about 50% of respective covered coated slides (Figure 4G,H).

Given the strong potency of UCNP1 and UCNP2 to inactivate coronavirus OC43, we extended this analysis to severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) with a genome of roughly 30 kb. Despite OC43 and SARS-CoV-2 belonging to the same family of viruses, treatment resulted in a differing extent of UCNP-mediated inactivation, presumably due to virus structural differences leading to disparate UV sensitivities. Approximately $10^5$ infectious PFUs of SARS-CoV-2 were delivered to untreated or UCNP1- or UCNP2-coated glass slides. Input virus infectivity was determined as time zero. Slides were then covered or exposed to light for 45 min, followed by a medium wash, and processed for remaining infectious SARS-CoV-2 (Figure 6). Untreated exposed samples maintained about $5 \times 10^3$ PFU/mL of infectious SARS-CoV-2, and light-exposed UCNP2-coated slides had about $10^2$ PFU/mL virus remaining (Figure 6). Remarkably, light-exposed UCNP1-coated slides reduced SARS-CoV-2 titers from $\sim 10^5$ PFU to undetectable levels of infectivity. Representative plaque assay images of the experiment are shown in Figure S9 (Supporting Information). Values are the mean of three independent sample replicates, with error bars representing the standard deviation and Un signifying undetectable virus infectivity. *** indicates p-value < 0.001 comparing untreated light-exposed slides to the UCNP-coated light-exposed slides.
2.6. UV-Light-Generating UCNP-Coated Slides Inactivated a Wide Range of Structurally Distinct Viruses.

Given that the susceptibility of a virus particle to UV-light-mediated inactivation can depend on the virion structure and type of genome, we tested the extent to which light-exposed UCNP-coated slides could inactivate a panel of structurally diverse viruses. Human rhinovirus RV14 and feline calicivirus (FCV) were used as prototype viruses to investigate the potency of UCNPs toward nonenveloped single-stranded positive-sense RNA viruses. RV14 has a genome size of approximately 7.5 kb, and the FCV genome is roughly 7.7 kb. Using the above inactivation assays, ∼10⁵ TCID₅₀ or PFUs of respective viruses were applied to untreated or UCNP-coated slides: Rhinovirus (RV14, panel A), FCV (panel B), PIV5 (panel C), VSV (panel D), Zika virus (panel E), or VV (panel F). Slides were covered (Cov) or exposed (Exp) to LED light for 1 h. Slides were then processed, and the remaining infectivity recovered from the slides was determined. Slides taken prior to light exposure are indicated as time 0. Values are the mean of three independent sample replicates, with error bars representing the standard deviation and Un representing undetectable infectivity. ** indicates p-value < 0.01 and *** indicates p-value < 0.001 comparing untreated light-exposed slides to the UCNP-coated light-exposed slides.

![Graphs showing viral titer (TCID₅₀/mL) over time for RV14, FCV, PIV5, VSV, Zika, and VV](https://doi.org/10.1021/acsami.2c11653)

**Figure 7.** UCNP1- and UCNP2-coated slides inactivate structurally distinct viruses when exposed to LED light. (A–F) Approximately 1 × 10⁵ TCID₅₀ or PFUs of respective viruses were applied to untreated or UCNP-coated slides: Rhinovirus (RV14, panel A), FCV (panel B), PIV5 (panel C), VSV (panel D), Zika virus (panel E), or VV (panel F). Slides were covered (Cov) or exposed (Exp) to LED light for 1 h. Slides were then processed, and the remaining infectivity recovered from the slides was determined. Slides taken prior to light exposure are indicated as time 0. Values are the mean of three independent sample replicates, with error bars representing the standard deviation and Un representing undetectable infectivity. ** indicates p-value < 0.01 and *** indicates p-value < 0.001 comparing untreated light-exposed slides to the UCNP-coated light-exposed slides.
A genome of about 11 kb. Approximately $10^5$ infectious PFU of each virus was delivered to glass slides that were untreated or treated with UCNP1 or UCNP2. Slides were then covered or exposed to LED light for 1 h, followed by a medium wash and processing for the remaining infectious virus. As shown in Figure 7C, light-exposed UCNP1-coated slides reduced PIV5 titers to $\sim10^7$ PFU/mL, and light-exposed UCNP2-coated slides completely inactivated PIV5 infectivity. Similarly, VSV was extremely sensitive to both light-exposed UCNP1 and UCNP2, with viral titers below detectable levels (Figure 7D).

The enveloped + ssRNA Zika virus with a genome size of $\sim11$ kb was tested for sensitivity to UCNP-mediated antiviral activity. After 1 h incubation of $\sim10^5$ infectious PFUs of Zika on untreated, UCNP1-, or UCNP2- treated glass slides, slides were washed in media, and samples were processed for remaining infectious virus (Figure 7E). Untreated exposed samples retained about $10^4$ PFU/mL of infectious Zika, whereas both light-exposed UCNP1- and UCNP2-coated slides reduced viral titers to undetectable levels.

All of the above-tested viruses have single-stranded RNA genomes, which are thought to be highly sensitive to UV treatment. From here, we tested the hypothesis that UCNP's coated slides could inactivate vaccinia virus (VV), a highly complex enveloped virus with a double-stranded DNA (dsDNA) genome $\sim190$ kb in length. Approximately $5 \times 10^5$ infectious PFUs of VV were applied to slides that were untreated or treated with UCNP1or UCNP2 and either covered or exposed to light for 1 h. Slides were washed in media and samples were processed for remaining infectious virus. As shown in Figure 7F, untreated exposed samples resulted in the recovery of $\sim10^4$ PFU/mL of infectious VV. Remarkably, both light-exposed UCNP1- and UCNP2-coated slides dramatically reduced viral titers to undetectable levels.

In considering results from viral deactivation studies for the different viruses by each UCNP formulation, trends in virus sensitivities may be generalized as responses to the unique emission properties of the particle formulations. Furthermore, the emission character and related virus sensitivities can potentially be tuned in future works by modifying the underlying, causative material structures. To understand the slight difference in efficacy between the two UCNP formulations, the differences in formulation UV emission character within both UVB and C should be considered, along with their related effects on biomolecules and viruses. Fluorescence spectra of UCNP1 and UCNP2 (shown in Figure 2) show a slight difference in their emission characteristics when excited with the same excitation source. Both formulations have emissions in the UVC (100–280 nm) and UVB (280–320 nm) ranges, but the main peak for UCNP1 in the UVC range is blue-shifted (shifted toward lower wavelength) compared to UCNP2. The UCNP2 formulation was observed to produce a broader emission range: with appreciable UVB range emission observed. UVB and UVC radiation-mediated damage to viruses has been discussed in Section 2.4. While UVC is generally regarded as producing a more substantial antimicrobial action, the combined effect of UVB and C emission is difficult to predict. To further understand the UV emission characteristics of the UCNP1 and UCNP2 formulations, we have conducted dye degradation experiments using AO16 dye, UCNPs, and LED light. Our initial results show that UCNP2 has better dye degradation characteristics. Methods followed and findings from dye degradation experiments are shown in Figure S7 (Supporting Information: 7. Reactive Orange 16 dye degradation using UCNP's).

SARS-CoV2, FCV, and PIV5 studies all showed disparate activities between UCNP1 and 2, suggesting varying susceptibilities to their unique emission characters. However, virus sensitivities were not specific to a given formulation with respect to enveloped, nonenveloped structure, genome size, or genetic material composition. These points taken together suggest a more complex relationship between virus character and UCNP emission character. It was observed, however, that both UCNP formulations produced substantial virus inactivation toward enveloped viruses as compared to nonenveloped viruses. This distinction implicates host cell recognition protein loss-of-function, loss of membrane integrity, and/or modification to genetic material as potential modes of viral inactivation. Conversely, the observed lower inactivation for the nonenveloped virus studies may occur as a result of physical shielding of the genetic material by the dense protein shell of the virion and/or the smaller genome size of the tested enveloped viruses, relative to the tested enveloped viruses. Further study into the specific mechanisms by which a given formulation imposes specific virus inactivation is part of our future work. In particular, damage to genetic material and viral proteins should be evaluated to identify formulation- and therefore emission character-specific antiviral processes.

Unlike chemical-based virucide, the activity of UCNP (1 and 2) depends only on the availability of white light, making it truly regenerative in nature. This character also makes the material suitable for self-cleaning coating applications on contamination-prone surfaces and PPEs. UCNPs were studied as drop-cast coatings as a proof of concept in the current study. In follow-up studies, we plan to incorporate these materials into more precisely designed architectures through a layer-by-layer (LBL) coating method. Preliminary experiments of this UCNP-LBL coating were conducted on glass and PPE (nitrile glove) surfaces; the results are shown in Figure S6. We have also performed a cytotoxicity (MTT cell viability assay) study of the UCNPs (Figure S4 and S5: Supporting Information). Normal phenotype MCF-7 and human umbilical vein endothelial cells (HUVECs) incubated at 24 or 48 h with UCNPs showed the fraction of viable cells as more than 90% (at up to 500 μg/mL) for both UCNP1 and UCNP2 samples, indicating negligible cytotoxicity.

3. CONCLUSIONS

Amid the COVID-19 pandemic, viral transmission is a major public health concern with substantial effects on the global economy and human societies. In the present study, we report visible-to-UV UCNPs based on self-cleaning, regenerative virucidal surfaces. Two formulations of yttrium silicates with Pr, Gd, and Li doping [UCNP1: Y$_2$SiO$_5$ (1.2% Pr, 1.2% Gd, and 7.2% Li); UCNP2: Y$_2$SiO$_5$ (1% Pr and 1% Gd)] were used for the upconversion of visible light to the UV C and B emission regions.

Antiviral studies of UV-light-generating UCNP-coated slides demonstrated abilities to inactivate structurally distinct viruses, with the virus structure being a strong indicator of susceptibility to UV-mediated inactivation. Generally, enveloped viruses, such as coronaviruses, seemed more susceptible to light-exposed UCNP-mediated inactivation as compared to nonenveloped viruses, such as RV14 and FCV. The nature of the viral genome did not correlate well with sensitivity to UCNP exposure, given that the dsDNA-containing VV was
among the most susceptible to inactivation, compared to the inactivation of the ssRNA-containing rhinovirus. However, our data indicate that the size of the viral genome may be an important factor in sensitivity to UCNPs since viruses with larger genomes (VV, PIV5, and VSV) were more sensitive to inactivation than those with smaller genomes (RV14 and FCV). Our characterization of UCNPs coatings, in the presented work, serves as a proof of concept for antiviral coatings on relevant surfaces.

### 4. MATERIALS AND METHODS

#### 4.1. Material Synthesis

**4.1.1. Materials.** Yttrium(III) nitrate hexahydrate, praseodymium(III) nitrate hexahydrate, gadolinium(III) nitrate hexahydrate, tetraethyl orthosilicate (TEOS), ethanol, lithium nitrate, and nitric acid (70%). All chemicals were >99.9% purity and procured from Sigma-Aldrich. DI water was used from a Barnstead DI water system.

**4.1.2. Experimental Procedure for UCNP Synthesis.** For the \(\text{Y}_2\text{SiO}_5\) \((1\% \text{Pr} \text{ and } 1\% \text{Gd})\) [UCNP2] sample, 8.809 g (0.023 mol) of \(\text{Y(NO}_3\)\)_3·6H\(_2\)O and 1.17 g (0.0027 mol) of \(\text{Pr(NO}_3\)\)_3·6H\(_2\)O were dissolved in 27.6 mL of anhydrous ethanol while the solution was kept at 70 °C for 5 min. 0.5 mL of 70% HNO\(_3\) and 1 mL of deionized (DI) water was added for partial hydrolysis. The solution was kept stirring for 5 min, then allowed to cool to room temperature, followed by the addition of 3.825 mL of TEOS. The solution was then heated to 70 °C in an oil bath and titrated with an additional 5.4 mL of DI water. Stirring was maintained until gel formation (approximately 30 min) followed by heating at 104 °C in a furnace for 17 h to form a xerogel. The product xerogel material was then ground using a mortar and pestle to decrease the size and then calcined at 1400 °C for 24 h. The final calcined material was mostly ground and sieved using 63, 45, and 25 μm pore-sized sieves, successively.

**4.1.3. UCNP-Coated Quartz Glass Slides.** Fused quartz glass slides, 14 × 14 × 1 mm, were procured from Skyline Components, LLC. UCNPs were dispersed in water using water bath sonication (BRANSON), 100 μg/mL concentration. The dispersed solution was drop-cast on the slides followed by drying, and the glass slides were weight was measured before and after drop casting. UCNPs were coated to approx. 2–3 μm thickness on the glass slides. Thickness was estimated using the weight difference between coated—uncoated slides, the density of \(\text{Y}_2\text{SiO}_5\) \((\sim 4.27 \text{ g/cm}^3)\), and coated area (14 × 14 mm).

**4.2. Material Characterization.**

**4.2.1. Characterizations of UCNP Formulations.**

**4.2.1.1. X-ray Diffraction.** XRD of UCNP powders was done using a PANalytical system with Cu-Kα radiation (1.54 Å) as the source. XRD data was analyzed using X’Pert Highscore software.

**4.2.1.2. X-ray Photoelectron Spectroscopy.** XPS was done using a Thermo Fisher EXCALAB-250 Xi spectrometer. UCNP samples were filled in the cavity of a sample holder designed for holding powder samples before putting the samples in an ultrahigh vacuum chamber (below 8 × 10⁻¹⁰ mbar). Monochromatic Al Kα radiation was used as the source with 300 W operating power, and 650 μm was the spot size of the beam. Thermo Avantage software was used for data analysis and peak fitting and deconvolution; C 1s peak (284.6 eV) was used for calibration.

**4.2.1.3. Transmission Electron Microscopy.** TEM was done using JEOL-1011 and FEI Tecnai F30 300 kV HRTEM systems. UCNPs were dispersed using a sonication bathe sonicator for 5 min. Monochromatic Al Kα radiation was used as the source. TEM was done using a TEM grid (SPI #3620C-MB). The powder was dispersed in water, followed by drop casting and drying (at room temperature) on the grid. Gatan digital micrograph software was used for image analysis. Virus TEM sample preparation was done using a negative staining procedure with phosphotungstic acid (99.9% pure, Sigma-Aldrich) as a negative strain. The procedure is explained in Figure S3, Supporting Information: Virus imaging by TEM.

**4.2.1.4. Fluorescence Spectroscopy.** A Hitachi F-7000 spectrometer was used to collect PL. A 488 nm light was used as the excitation.
source, and emission was recorded over a range (260 to 400 nm). The emission and excitation slit width was set at 5 nm.

4.3. Experimental Setups. 4.3.1. Light Source and Radiometer. We purposefully used a common LED light (Ustarlight 60W LED work light) for our experiments. For control experiments, we used 254 nm UV light (UVP EL Series 4-Watt UV lamps) with a stand of 10.74 in. height (Thermo Fisher: UVP 18006301). We used a radiometer (Thermo Fisher: UVP UVX radiometer) with a 254 nm sensor (Thermo Fisher: UVX-25 sensor) for spectral measurement.

4.3.2. Cells, Viruses, and Infectivity Assays. Cultures of HCT-8 cells were grown in a Roswell Park Memorial Institute (RPMI 1640, Gibco, Thermo Fisher Scientific) supplemented with 10% heat-inactivated fetal calf serum (HI FBS, Gibco, Thermo Fisher Scientific). Cultures of RD, VeroE6, HeLa, Crandell–Rees feline kidney (CRFK), Vero, and CV-1 cells were grown in Dulbecco’s modified Eagle’s medium (DMEM, Gibco, Thermo Fisher Scientific) supplemented with 10% HI FBS.

Human Coronavirus OC43 (ATCC, catalog number VR-1558) was grown in HCT-8 cells at 33 °C. The medium from virus-infected cells was clarified by centrifugation and aliquots were quickly frozen for storage at −80 °C. OC43 stocks were quantified using a standard 50% tissue culture infectious dose assay (TCID50) on confluent RD cells in 96-well plates (Falcon, Thermo Fisher Scientific). In brief, solutions were serially diluted in DMEM containing 0.38% bovine serum albumin as a carrier protein. Cells were washed with PBS and incubated with diluted virus solutions for 1 h at 33 °C. Cells were then washed and replaced with DMEM containing 2% HI FBS. After 4 days (d) post-infection (DPI) at 33 °C, cells were washed with PBS and stained with a crystal violet solution as described previously. TCID50 were calculated by the Spearman and Kärber algorithm as previously described.

The USA-WA1/2020 strain of SARS-CoV-2 was kindly provided by Ashley Brown, University of Florida and originally obtained from BEI Resources (catalog number NR52281 and NR-52285, Source Centers for Disease Control and Prevention). SARS-CoV-2 was grown in VeroE6 cells at 37 °C under BSL3 protocols approved by the UCF Biosafety Committee. At 3 DPI, the medium was clarified by centrifugation, and aliquots were frozen for storage at −80 °C. SARS-CoV-2 titers were determined by the plaque assay on confluent VeroE6 monolayers using 0.4% agarose and DMEM containing 2% HI FBS at 37 °C. After 3 dpi, cells were stained with 1% crystal violet as described previously.

Human rhinovirus 14 (RV14, ATCC, catalog number VR-284) was grown in HeLa cells at 33 °C. Media and dislodged cells were combined by centrifugation, followed by three rounds of freeze/thaw, clarification by centrifugation, and aliquots were quickly frozen and stored at −80 °C. RV14 stocks were determined by a TCID50 assay using confluent HeLa cells in 96-well plates. FCV (ATCC, catalog number VR-782) was grown in CRFK cells (ATCC, catalog number CCL-94) at 37 °C. At d1pi, media and dislodged cells were harvested and virus stocks were prepared as described for RV14. FCV virus titer were determined by the plaque assay on CRFK cells.

Wild-type PIV5 (WT PIV5) encoding the bacterial flagellin gene inserted between the HN and L genes was grown in Vero cells. Viruses were concentrated by centrifugation through a glycerol cushion, and titers were determined by the plaque assay on CV-1 cells. VSV (strain Orsay), Zika virus (strain MR766), and VV(WR strain) were grown at 37 °C in BHK-1, Vero, or CV-1 cells, respectively. The medium was clarified by centrifugation, and aliquots were quickly frozen for storage at −80 °C. Virus titers were on confluent Vero (VSV, Zika) or CV-1 (VV) monolayers.

4.3.3. Virus Inactivation Studies. Uncoated or UCNP-coated quartz glass slides were prepared as described above and used within 3 days of preparation. Approximately 1 × 10^6 TCID50 or PFUs of each respective virus were delivered to slides in triplicate in the dark in a biosafety hood. Titors of virus recovered as initial input infectivity were determined. Control slides were covered with tin foil, and exposed samples were exposed to a LED light (Ustarlight 60W LED work light) at a distance of 5 inches from the slides. At the incubation times indicated in the figures, slides were vortexed and washed in an assay tube containing 0.5 mL of DMEM and 2% HI FBS. Samples were then analyzed for infectivity according to respective virus quantification assays as described above.

4.3.4. Reverse Transcription (RT-PCR). Control and LED light-exposed UCNP-coated quartz glass slides were prepared as described above and inoculated with approximately 1 × 10^7 TCID50 of OC43. Slides were exposed as described above; media wash was then processed for RNA extraction using TRIzol (Invitrogen, Carlsbad, CA, USA). To produce cDNA, 0.25 μg of total RNA was used in reactions with TaqMan reverse transcription reagents (Applied Biosystems, Foster City, CA, USA) as per the manufacturer’s instructions. Primers designed to amplify the OC43 Spike gene were purchased from Integrated DNA Technologies (IDT, Coralville, IA, USA). The forward primer sequence utilized 5′-ACAGACAGCTGCAACCCATTA-3′ and the reverse primer sequence utilized 5′-GCGATGGAAGCAACATTTGG-3′. RT-PCR was performed using GoTaq G2 Hot Start Green Master Mix (catalog number M7422, Promega, Madison, WI, USA) according to the manufacturer’s instructions. PCR products were run on a 1% agarose gel and visualized using a UV light source (Ultra Lum, model number UVB-10, Carson, California, USA). Band intensities were measured using ImageJ [Image] version 1.52a (National Institutes of Health, USA); http://imagej.nih.gov/ij/ (Rasband). Background measurements were subtracted from all samples. Exposed samples were then normalized to the corresponding covered sample and expressed as a percentage of the covered sample.

4.3.5. Statistical Analyses. Values are the mean of three independent samples, with error bars representing the standard deviation. Statistical analysis was performed using GraphPad unpaired student T-test comparing untreated light-exposed slides to the UCNP-coated light-exposed slides. In all figures, * indicates p-value < 0.05, ** indicates p-value < 0.01, and *** indicates p-value < 0.001.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsami.2c11653.

PL spectrum of UCNPs and size distribution estimation using TEM image; doped yttrium silicate formulations’ thermal behaviors: material composition and structural change/stability; virus imaging by TEM; UV light dose required to inactivate coronavirus; cytotoxicity of UCNP; LBL coating developed on a glass substrate and PPE materials; reactive Orange 16 dye degradation using UCNP; and representative images of plaque and TCID50 assays for viruses tested (PDF)

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PL spectrum of UCNPs and size distribution estimation using TEM image; doped yttrium silicate formulations’ thermal behaviors: material composition and structural change/stability; virus imaging by TEM; UV light dose required to inactivate coronavirus; cytotoxicity of UCNP; LBL coating developed on a glass substrate and PPE materials; reactive Orange 16 dye degradation using UCNP; and representative images of plaque and TCID50 assays for viruses tested (PDF)
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Author Contributions

U.K. and C.R.F. have contributed equally. Conceptualization: U.K., C.J.N., S.S., E.K., and G.D.P.; investigation: U.K., C.R.F., E.K., K.K., and G.D.P.; formal analysis: U.K., C.R.F., E.K., C.J.N., and Y.F.; supervision: S.S. and G.D.P.; visualization and writing: U.K., C.R.F., E.K., C.J.N., K.K., Y.F., E.M., B.B., G.D.P., and S.S.

Notes

The authors declare no competing financial interest.

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