Inactivation of SARS-CoV-2 and Other Enveloped and Non-Enveloped Viruses with Non-Thermal Plasma for Hospital Disinfection

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ABSTRACT: As recently highlighted by the SARS-CoV-2 pandemic, viruses have become an increasing burden for health, global economy, and environment. The control of transmission by contact with contaminated materials represents a major challenge, particularly in hospital environments. However, the current disinfection methods in hospital settings suffer from numerous drawbacks. As a result, several medical supplies that cannot be properly disinfected are not reused, leading to severe shortages and increasing amounts of waste, thus prompting the search for alternative solutions. In this work, we report that non-thermal plasma (NTP) can effectively inactivate SARS-CoV-2 from non-porous and porous materials commonly found in healthcare facilities. We demonstrated that 5 min treatment with a dielectric barrier discharge NTP can inactivate 100% of SARS-CoV-2 (Wuhan and Omicron strains) from plastic material. Using porcine respiratory coronavirus (surrogate for SARS-CoV-2) and coxsackievirus B3 (highly resistant non-enveloped virus), we tested the NTP virucidal activity on hospital materials and obtained complete inactivation after 5 and 10 min, respectively. We hypothesize that the produced reactive species and local acidification contribute to the overall viricidal effect of NTP. Our results demonstrate the potential of dielectric barrier discharge NTPs for the rapid, efficient, and low-cost disinfection of healthcare materials.

KEYWORDS: non-thermal plasma, surface disinfection, virus inactivation, SARS-CoV-2, virucidal

INTRODUCTION

The early 21st century has now experienced two epidemics caused by new coronaviruses, SARS-CoV-1 (2003) and MERS-CoV (2012 to present), and a pandemic caused by SARS-CoV-2 (2019 to present).1 As reported by the World Health Organization (WHO), there have been more than 640 million confirmed cases of COVID-19 globally, including more than 6.5 million deaths to date.2 In addition to dramatic loss of human lives worldwide, the economic and social disruption caused by the pandemic presents an unprecedented challenge to public health.3 It has been demonstrated that SARS-CoV-2 can survive on porous materials for up to 2 days,4 and on non-porous surfaces for up to 7 days, turning them into potential sources of infection.5 To prevent the spread of the virus, hospitals are forced to dispose of several tons of contaminated hospital materials that could not be disinfected for safe reuse during the pandemic,6 thus increasing the generation of waste around the world by 400–500%.7 This led to severe shortages of medical supplies with drastic consequences for patient care.8 As rightly pointed out by the WHO, the enormous and ever-increasing quantity of hospital waste represents a major environmental challenge, even outside of pandemic periods.9

The current disinfection technologies are not suitable for a broad range of materials urgently required in the hospital settings. Thermal disinfection requires long cycles (>60 min) and is not compatible with moisture- and heat-sensitive materials. Chemical disinfectants are more adapted to heat-sensitive medical items, but they are potentially toxic, flammable, or corrosive.10 This includes low-level disinfectants that can destroy bacteria and some viruses, but not bacterial spores (e.g., sodium hypochlorite) and high-level disinfectants that can also kill bacterial spores (e.g., ethylene oxide and chlorine dioxide).11 Furthermore, shortages of these chemicals...
have become an issue in times of high demand, as experienced by hospitals during the pandemic.\textsuperscript{12} The use of ultraviolet (UV) radiation to inactivate pathogens has been an attractive alternative, but only surfaces exposed to the path of the UV light can be disinfected.\textsuperscript{13} The environmental and economic impact of healthcare waste evidenced by the pandemic has encouraged the search for alternative easy-to-use, efficient disinfection techniques.\textsuperscript{14}

In the past years, non-thermal plasma (NTP), a novel method that combines chemical and physical reactions,\textsuperscript{15} has proven to be an attractive alternative for disinfection. NTP is a partially ionized gas that can be created at room temperature and atmospheric pressure. By solely using air, and without additional harmful chemicals, NTP creates highly reactive oxygen and nitrogen species (RONS; e.g., $\cdot$OH, O$_2$•$, NO$, H$_2$O$_2$, and ONOO$^-$) which can rapidly interact with, destroy, or inactivate biological cells and pathogens.\textsuperscript{16} It is well accepted that the rich cocktail of RONS is the main factor driving decontamination, and the UV photons and electromagnetic fields produced by NTP contribute to the generation of RONS.\textsuperscript{17} NTP has been broadly demonstrated to inactivate bacteria and a broad range of viruses on different matrices, mainly due to its action on capsid proteins and nucleic acids.\textsuperscript{18,19} These properties of NTP make them an attractive, environmentally friendly solution for disinfection of moisture- and temperature-sensitive supplies. Currently, there are two main categories of NTP devices for disinfection: (1) plasma jets and (2) direct dielectric barrier discharges (DBDs).\textsuperscript{20} In plasma jets, the plasma is generated remotely with a feed gas (e.g., argon, helium, and gas mixtures) and delivered to the target via the gas flow and ionization waves.\textsuperscript{21} On the other hand, direct DBD devices generate NTP directly onto the surface they are treating, using atmospheric air.\textsuperscript{22} For viral disinfection, additional gas flow could further spread viral particles, and therefore, plasma jets are ill-adapted for this application. Therefore, DBD NTP devices are better adept for mitigation of contagious virus and surface disinfection.

The aim of this study is to demonstrate the potential of a DBD NTP device to inactivate SARS-CoV-2 from hospital materials, such as plastics and fabrics. For this purpose, we have characterized the inactivation of SARS-CoV-2 Wuhan and Omicron variants from plastic materials and used the porcine respiratory coronavirus (PRCV) and coxsackievirus B3 (CVB3) as safer virus models to evaluate NTP virucidal activity from porous fabrics. Our results demonstrate that NTP can effectively inactivate SARS-CoV-2 and more resistant viruses from materials commonly found in healthcare facilities. This study provides fundamental insights into the virucidal action of NTP, while supporting its value for the development as a hospital disinfection tool. Translation of this more sustainable technology would support the supply shortage, environmental impact, and healthcare consequences of future pandemics while reducing the amount of waste produced by hospitals in general.

\section*{MATERIALS AND METHODS}

\textbf{NTP Inactivation Experiments.} \textit{NTP Source.} NTP was generated using a microsecond-pulsed DBD plasma system (Figure 1a) previously described.\textsuperscript{23} Briefly, the power supply was custom-built (Megaimpulse Ltd., Russia), producing a 2 $\mu$s pulse width (30 kV) with a rise time of 1–1.5 $\mu$s. The output of the power supply was connected to a DBD electrode. The DBD copper electrode (3 mm diameter) was covered with a 0.5 mm fused silica dielectric (Technical Glass). The frequency of the pulses was fixed to 1000 Hz for all experiments. The working distance, measured between the bottom of the DBD electrode and the top of the material on which the virus suspension was deposited, as well as the treatment time, were optimized for each material to obtain virus inactivation.
Cell Culture and Virus Stocks. Vero (ATCC CCL-81) and swine testicular (kind gift of Prof. Nauwynck, Ghent University) cells were routinely maintained in Dulbecco’s modified Eagle’s medium (DMEM, Thermo Fisher Scientific) supplemented with 10% heat-inactivated fetal bovine serum (iFBS) and 2% of penicillin-streptomycin (P–S). Cells were routinely incubated at 37 °C in a CO₂ atmosphere with 95% humidity. SARS-CoV-2 Wuhan strain (lineage B Wuhan-Hu-1, 2019-nCoV-Italy-INM11, reference 008V-03893), SARS-CoV-2 Omicron B.1.1.529.1 (BA.1) variant (strain VLD20211207: isolated and cultured at the Institute of Tropical Medicine of Antwerp), human coxsackievirus B3 (ATCC VR-30), and porcine respiratory coronavirus (PRCV; strain 91V44, a kind gift of Dr. H. Nauwynck, Ghent University, Belgium) were used. SARS-CoV-2 and coxsackievirus (CVB3) were grown and titrated in Vero cells. PRCV was grown and titrated in swine testicular (ST) cells. Infectious virus titers of SARS-CoV-2 and PRCV were determined by the median tissue culture infectious dose assay (TCID₅₀), calculated by the Reed–Muench method and expressed as log₁₀ TCID₅₀/mL. Infectious virus titer of CVB3 was determined by the plaque forming unit (PFU) assay and expressed as log₁₀ PFU/mL. The titers of the virus stocks were ≈ log₁₀ TCID₅₀/mL for SARS-CoV-2 Wuhan strain, ≈ log₁₀ TCID₅₀/mL for SARS-CoV-2 Omicron variant, ≈ log₁₀ TCID₅₀/mL for PRCV, and ≈ log₁₀ PFU/mL for CVB3 and were directly used without dilution for NTP experiments.

NTP Treatment of Virus Suspension on Plastic Material. Viral inactivation on plastic material was done in polystyrene culture plates. A volume of 25 μL of virus suspension was deposited in the center of a well (662102 Greiner Bio-One CELLSTAR cell culture plate, 24-well, flat bottom) and immediately treated with the DBD with a frequency of 1000 Hz, and an optimal working distance of 2 mm (Figure 1b). NTP treatments were carried out for 5 min for SARS-CoV-2 and PRCV and 10 min for CVB3. Immediately after the NTP treatment, 100 μL of DMEM (2% iFBS, 2% P–S) was added to rinse the well and to allow for virus recovery. The total volume was collected and used for titration. The evaporation due to NTP treatment was taken into account to obtain corrected values of infectious titer per volume. A non-treated virus suspension (25 μL) was processed similarly and used as a negative control. All measurements were performed with at least three biological replicates and three independent replicates.

NTP Treatment of Virus Suspension on Porous Hospital Materials. Hospital pillowcase (fabric 1) and hospital gown (fabric 2) were selected as representative porous hospital materials. Fabric 1 was made of 100% cotton fibers and fabric 2 was made of a blend of cotton and synthetic polyester. Hospital fabrics were cut in 1 cm² pieces and immersed in 90% ethanol solution for cleaning. The samples were rinsed in Milli-Q water and allowed to dry overnight in a safety cabinet. The sample were positioned in the center of a well (662102 Greiner Bio-One CELLSTAR cell culture plate, 24-well, flat bottom) and 25 μL of virus suspension was deposited at the surface of the sample. After 30 min of incubation, the sample was treated with the DBD with a frequency of 1000 Hz, an optimal working distance of 1 mm and during 5 min for PRCV and 10 min for CVB3 (Figure 1c). Immediately after the NTP treatment, 200 μL of DMEM (2% iFBS, 2% P–S) was added in the well to cover the sample and the plate was placed in an orbital shaker (150 RPM) for 30 min for virus recovery. In comparison with the treatment on the plastic plate, the volume to rinse the well was increased (from 100 to 200 μL) to ensure a full immersion of the sample in the liquid. The total volume in the well was collected and used for titration. A non-treated virus suspension (25 μL) was processed similarly and used as a negative control. All measurements were performed with at least three biological replicates and three independent replicates.

TCID₅₀ Titration of SARS-CoV-2 and PRCV. The titer of both strains of SARS-CoV-2 was assessed following the TCID₅₀ method in Vero cells in a 96-well format. Each recovered sample was subjected to 10-fold serial dilutions and incubated in 4-fold with freshly plated 1.8 × 10⁴ Vero cells for 1 week (37 °C, 5% CO₂). After 7 days, the wells were examined microscopically for the presence of the viral cytopathic effect caused by viral growth, and the virus titer was calculated using the Reed–Muench method.

The titer of PRCV samples was assessed following the TCID₅₀ method in ST cells in a 96-well format. Each recovered sample was subjected to 10-fold serial dilutions and incubated in 6-fold with 1 day old plated 2 × 10⁴ ST cells in 96-well plates for 1 week (37 °C, 5% CO₂). After 6 days, the presence of the viral cytopathic effect was microscopically evaluated and the virus titer was calculated using the Reed–Muench method. The detection limits are 2.4 log₁₀ TCID₅₀/mL for SARS-CoV-2 and 1.3 log₁₀ TCID₅₀/mL for PRCV.

CVB3 Plaque assay. The titer of CVB3 samples was assessed in Vero cells in a 6-well format. Each recovered sample was subjected to 10-fold serial dilutions and incubated in 4-fold with 1 day old plated 1.3 × 10⁴ Vero cells for 1 h (37 °C, 5% CO₂) with agitation every 15 min. The viral medium was aspirated and the infected cell layer was covered by a 0.6% Avicel solution with DMEM (10% iFBS, 2% P–S, 3 mL/well) and incubated for 2 days (37 °C, 5% CO₂). Avicel overlay was removed and the cells were fixed with 4% paraformaldehyde solution and stained with 0.25% crystal violet. The number of visible plaques caused by viral growth was determined and used for PFU titer calculation. The detection limit was 1.9 log₁₀ PFU/mL.

Thermal Imaging. Thermal images were recorded using a cooled FLIR x6540sc thermal imaging camera during NTP treatment of 25 μL of DMEM (2% iFBS, 2% P–S) in a 24-well plate. The camera has an InSb detector with a resolution of 640 × 512 pixels, with a measurement accuracy of ±1 °C, and a thermal sensitivity/noise equivalent temperature difference <25 mK. Measurements were carried out without filter and using an L1206 50 mm f = 3 lens, which has a spectral range of 1.5–5 μm. All NTP discharges were observed with a frame rate of 30 fps and the image sequences were recorded using FLIR Researcher IR Max software. Afterward, all data were processed in MathWorks MATLAB. The data were obtained and processed at the Industrial Vision Lab (InViLab), University of Antwerp.

pH Measurement. The DBD was used to treat 25 μL DMEM (2% iFBS, 2% P–S) in a 24-well plate. The working distance was 2 mm and NTP was generated at a 1000 Hz pulse frequency and varying treatment times. The remaining volume was collected in Eppendorf tubes directly after the treatment and the pH was analyzed in 30 min following the treatment with a custom-made pH microprobe ("Leak Free" 1 mm pH probe AMANI 1000L, Harvard Apparatus) from the Applied Electrochemistry & Catalysis (ELCAT) research group from the University of Antwerp, in a minimum volume of 10 μL. All measurements were performed at least three times on three independent replicates.

RONs Quantification. The DBD NTP system was used to treat 25 μL DMEM (2% iFBS, 2% P–S) in a 24-well plate. The working distance was 2 mm and NTP was generated at a 1000 Hz pulse frequency and varying treatment times. Following the treatment, 1 mL of (2% iFBS, 2% P–S) was immediately added to rinse the well. The total volume was collected and analyzed. The evaporation of treated medium due to NTP treatment was measured for each treatment time and taken into account to obtain the corrected values of RONS concentration. All chemical measurements were performed at least three times on three independent replicates.

For the quantification of NO₂⁻ and NO₃⁻, a fluorometric assay kit (780051; Cayman Chemical) was used according to the manufacturer’s instructions. To measure both NO₂⁻ and NO₃⁻ (NO₂⁻ + NO₃⁻), 20 μL of 20X diluted samples was added in a 96-well plate and the volume was adjusted to 80 μL using DMEM (2% iFBS, 2% P–S). A nitrate reductase mixture (780010; Cayman Chemical) and an enzyme cofactor mixture (780012; Cayman Chemical) were added to each well and incubated for 1 h, allowing for the conversion of NO₃⁻ into NO₂⁻. DAN reagent (780070; Cayman Chemical) provided as an acidic solution was added to each well and incubated for 10 min before adding NaOH (780068; Cayman Chemical), which enhances the detection of the fluorescent product 1H-naphthotriazole. The plate was read with the Tecan Spark Cyto (λₐex: 365 ± 20 nm, λₑm: 430 ± 20 nm, fixed gain: 64). To measure NO₂⁻, 20 μL of 10X diluted samples was added in a 96-well plate and the volume was
adjusted to 100 μL using DMEM (2% iFBS, 2% P−S). DAN reagent (780070; Cayman Chemical) was directly added to each well and incubated for 10 min before adding NaOH (780068; Cayman Chemical) and reading the plate with Tecan Spark Cyto (λ\text{ex}: 365 ± 20 nm, λ\text{em}: 430 ± 20 nm, fixed gain: 81). An estimate of NO\textsubscript{3}− concentrations was, therefore, calculated by subtracting the mean NO\textsubscript{2}− concentration of each treatment condition from the NO\textsubscript{3}−+NO\textsubscript{2}− concentration. Concentrations of NO\textsubscript{3}− and NO\textsubscript{2}− were calculated from a calibration curve, obtained using standard solutions provided in the assay kit.

For the quantification of H\textsubscript{2}O\textsubscript{2}, a fluorometric assay kit (MAK165; Merck) was used according to the manufacturer’s instructions. A volume of 50 μL of 10× diluted samples (in DMEM 2% iFBS, 2% P−S) was added in a 96-well plate and mixed with 50 μL of a master mix containing 4.75 mL of assay buffer + 50 μL of red peroxidase substrate + 200 μL (20 units/mL) peroxidase. The samples were incubated for 30 min and the fluorescence was measured using the Tecan Spark Cyto (λ\text{ex}: 540 ± 20 nm, λ\text{em}: 590 ± 20 nm, fixed gain: 54). Concentrations of H\textsubscript{2}O\textsubscript{2} were calculated from a calibration curve, obtained using standard solution provided in the assay kit.

Statistical Analysis. Student’s t-test was performed using Prism v9.3.1 (GraphPad Software, San Diego, CA, USA). Statistical significance was set at p ≤ 0.05.

RESULTS

SARS-CoV-2 Inactivation on Plastic Material. Two strains of SARS-CoV-2 were treated with NTP on plastic plates. For the SARS-CoV-2 Wuhan strain, 5 min of NTP treatment reduced the initial infectious titer by 4.9 log\textsubscript{10} (6.5−1.6 log\textsubscript{10} TCID\textsubscript{50}/mL; Figure 2a). This reduction was equivalent to a complete inactivation since on average, the obtained infectious titer after treatment (1.6 log\textsubscript{10} TCID\textsubscript{50}/mL) was below the detection limit of the titration method (2.4 log\textsubscript{10} TCID\textsubscript{50}/mL). For the SARS-CoV-2 BA.1 Omicron variant, NTP completely inactivated the viral sample after 5 min of treatment (Figure 2a). These results demonstrate that NTP can effectively inactivate SARS-CoV-2 from plastic material.

Virus Models’ Inactivation on Porous Hospital Materials. Once NTP was demonstrated to inactivate both the Wuhan and Omicron strains of SARS-CoV-2, PRCV and CVB3 were used as virus models to investigate NTP disinfection of porous hospital materials, as these viruses could be more easily handled in BSL-2 facilities. The response of the virus models to NTP was preliminarily assessed on a plastic plate in the same way as for SARS-CoV-2. NTP completely inactivated PRCV after 5 min of treatment (with ≥5.8 log\textsubscript{10} PFU/mL reduction; Figure 2b) and CVB3 after 10 min of treatment (with ≥7.5 log\textsubscript{10} PFU/mL reduction; Figure 2c). Altogether, these data demonstrate that NTP can effectively inactivate SARS-CoV-2 from plastic material.

We then assessed viral inactivation from porous hospital materials using fabrics from hospital bed pillowcases (fabric 1)
and hospital gowns (fabric 2), artificially contaminated with PRCV and CVB3. For PRCV, NTP completely reduced the initial infectious titer after 5 min of treatment for both fabrics (fabric 1: $\geq 3.9 \log_{10} \text{TCID}_{50}$/mL reduction; fabric 2: $\geq 4.2 \log_{10} \text{TCID}_{50}$/mL reduction; Figure 2d). For CVB3, NTP achieved complete reduction of the initial infectious titer after 10 min of treatment (fabric 1: $\geq 7.2 \log_{10}$ PFU/mL; fabric 2: $\geq 7.5 \log_{10}$ PFU/mL; Figure 2e).

Our results demonstrate that NTP can inactivate PRCV using the same conditions as for SARS-CoV-2, possibly due to the direct effect of RONS on the lipid envelope, which affects the viral infectivity. Thus, PRCV is a suitable surrogate virus to assess SARS-CoV-2 viral disinfection with NTP. On the other hand, CVB3 appeared to be more resistant to NTP. The inactivation of non-enveloped viruses such as CVB3 requires the denaturation of the capsid proteins and damage to the RNA, which makes them more resistant to disinfection, as shown here.

**Effect of Temperature and pH on NTP Viral Inactivation.** To investigate the underlying mechanisms of NTP-based viral inactivation, we measured the evolution of the local temperature at the tip of the DBD electrode (Figure 3a, white arrow) continuously during NTP treatment of the different samples (plastic, fabrics 1, and fabric 2; Figure 3b). During the first 30 s following NTP initiation, the temperature was increased by 6 to 10°C for the different samples, with the local temperature never exceeding 35°C, even after 10 min of treatment. Therefore, it was clear that NTP treatment temperature was not responsible for its virucidal effects.

As low pH is also known to denature viruses and NTP has acidification effects, we assessed the pH of NTP-treated medium at the equivalent treatment times and conditions used for viral inactivation. Our results demonstrate that the pH decreased over the course of treatment (Fig. 3-c), reaching pH = 4 after 5 min of treatment and pH = 1.6 after 10 min. Therefore, it is possible that the acidification of the liquid solution contributes to the mechanism of viral inactivation by NTP treatment.

**NO$_3^-$, NO$_2^-$, and H$_2$O$_2$ Quantification.** To determine the role of the reactive species present in the NTP-treated solutions, we assessed the concentrations of NO$_3^-$, NO$_2^-$, and H$_2$O$_2$ in NTP-treated medium (without virus) at different treatment times. The concentrations of NO$_3^-$ and H$_2$O$_2$ were time-dependent, with longer exposure times leading to higher concentrations (Figure 4a,c). After exposure to NTP, NO$_3^-$ and H$_2$O$_2$ were measured to be 14 ± 2.78 and 2 ± 0.23 mM, respectively, at 5 min and 38 ± 10.4 and 3 ± 0.38 mM, respectively, at 10 min. Interestingly, NO$_2^-$ concentrations demonstrated a similar behavior up to 5 min (4 ± 0.47 mM), before dropping for 10 min of treatment (1.5 ± 0.60 mM; Figure 4b). We speculate that the antiviral activity of NTP is partially mediated by the effect of the reactive species measured here. However, we acknowledge that other relevant NTP-induced reactive species could be present in the treated medium such as peroxynitrite (ONOO$^-$), but were not measured in this study.

**DISCUSSION**

The recent SARS-CoV-2 pandemic has highlighted the need for new technologies to disinfect clinical settings and materials. Not only is this required for proper care of patients and healthcare workers, but it is also needed to reduce supply shortages, healthcare costs, and waste production. In this study, we used NTP technology to inactivate SARS-CoV-2 (the most relevant Wuhan and Omicron strains), PRCV, and CVB3 from porous and non-porous hospital materials. We demonstrated that the DBD is able to completely inactivate SARS-CoV-2 and surrogate viruses after only 5–10 min of treatment. Compared to hospital room surfaces, which have been reported to have the presence of 0.1 to 102 SARS-CoV-2 RNA genome copies per square centimeter (gc/cm$^2$), the viral loads used in our work were significantly higher: 500,000 times higher for the Wuhan strain and 1000 times higher for the Omicron variant. This further demonstrates the ability of NTP technology to rapidly and effectively inactivate high viral loads from various hospital materials (log 5 reduction) and complies with the requirements of the European Union standards for virus-inactivating disinfectants (reduction > log 4). Our results also provide insights into the efficacy of NTP against viruses and highlight the potential of NTP for surface decontamination.

In the present work, we used enveloped (SARS-CoV-2 and PRCV) and non-enveloped (CVB3) viruses to test the antiviral activity of our DBD plasma system. Both SARS-CoV-2 and PRCV are enveloped RNA viruses that belong to the *Coronaviridae* family and present morphological, biophysical, and genomic similarities, which makes PRCV a suitable surrogate virus model for SARS-CoV-2. The parameters required for NTP inactivation of SARS-CoV-2 and the surrogate virus PRCV were similar, while longer treatment times were needed for the CVB3 virus. CVB3, a human non-enveloped RNA enterovirus, is more resistant to common disinfection methods and to environmental stressors, such as desiccation and temperature changes, compared to that of enveloped viruses. Viral inactivation of both virus types via...
RONS is mediated by oxidative damage to proteins, viral envelopes (when present), and nucleic acids.\(^{35}\) For example, it has been demonstrated that NTP-derived RONS damage the receptor-binding domain of the spike (S) protein of SARS-CoV-2, key for its anchorage to host cells, thus reducing its ability to infect cells.\(^{35}\) These RONS can also degrade the viral RNA from aerosols and surfaces.\(^{36,37}\) NTP produces a wide variety of RONS with antiviral properties, such as singlet oxygen (\(\text{O}_2^\cdot\)), hydroxyl radical (\(\cdot\text{OH}\)), superoxide (\(\text{O}_2^\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\·
million tons of plastic waste per year from both used and unused medical supplies that cannot be reused or recovered. This represents an environmental and financial challenge for the healthcare systems. The amount of medical waste and the associated costs are remarkable and highlights the need for better disinfection tools suitable for a broad range of materials. In this context, DBD NTP technology could be a suitable tool to disinfect hospital supplies. In addition, solutions like DBD NTPs could be part of the prevention strategies to strengthen the global preparedness against future public-health crises.

The WHO guidelines for disinfection provide a list of conditions that an ideal disinfectant must: (1) have a high germicidal activity against a wide range of microorganisms, (2) be chemically stable, (3) be effective in the presence of organic compounds, (4) be compatible with the surface being disinfected, (5) be able to penetrate into crevices (desirable), and (6) be inexpensive and aesthetically acceptable. NTP satisfies these proposed conditions, compared to many current state-of-the-art devices and methods. The rapid and complete inactivation obtained for the viruses on porous and non-porous materials reveals the high efficiency of DBD NTP in inactivating not only SARS-CoV-2 but also highly resistant human viruses from hospital materials. The advantage of the DBD NTP source is that it does not require the addition of chemicals or components, as it uses atmospheric air, thus making it operationally inexpensive. In addition, the low temperatures and short treatment times further make DBD NTP an attractive technology for decontamination. In DBD NTP sources, only the electricity costs for powering the device have to be accounted for, but NTP is ideally suited to be combined with renewable electricity due to its short switch on/off times. Other studies have reported viral inactivation with different NTP devices but not without drawbacks. The need of vacuum systems, feed gases high gas temperatures that can damage the materials, or very long treatment times make those devices unsuitable for routine hospital use. Moreover, gas-fed NTP, such as plasma jets, could present additional health risks, as the constant gas flow could blow microdroplets that carry viruses and other contaminating particles into the surrounding environment, thus further spreading its reach. Another strategy which uses NTP for disinfection is via generation of NTP-treated liquids, such as water.

In this modality, NTP is used to treat and enrich a liquid with RONS. This liquid is then applied to the surface to be disinfected. However, a major drawback is that it can only be used on wettable surfaces. This drawback is similar to the use of super-oxidized water, where an electrical current is passed through salt water to generate species such as hypochlorous acid, dissolved oxygen, superoxide radicals, and more. Furthermore, the time between NTP liquid treatment and liquid application to the surface is critical, as several RONS, particularly the short-lived RONS, are unstable over time. Interestingly, Guo et al., has reported that O2, a short-lived RONS, could have an important function in bacteriophage inactivation with NTP-treated water, thus further highlighting the importance of RONS stability in NTP-treated liquids. If there is a significant delay between NTP enrichment of the liquid and application to the surface for disinfection, then the NTP-treated liquid is nothing more than a combination of the stable RONS (e.g., H2O2, NO−, and NO2−), which can easily be made through commercial products. DBD NTP technology does not have the drawbacks of plasma jets and NTP-treated liquids and therefore is most ideal for inactivation of contagious viruses, such as SARS-CoV-2, from medical products that cannot be decontaminated otherwise in a safe manner. Direct generation of DBD onto the surface for disinfection guarantees the delivery of the potent, short-lived RONS (e.g., singlet oxygen, atomic oxygen, and hydroxyl radicals). We believe that large-scale DBD NTP devices for disinfection of hospital materials are novel, sustainable solutions to help reduce costs and waste production, for both future pandemics as well as routine daily practice. However, there are several parameters that must be optimized, such as NTP treatment time. This is partially dependent on the material being disinfected, as there requires a balance between adequate viral inactivation and preventing damage to treated materials. Research into new geometric designs and scalability is needed and is currently ongoing in our lab.

**CONCLUSIONS**

In this work, we successfully used NTP technology to inactivate SARS-CoV-2, PRCV, and CVB3 from porous and non-porous materials commonly found in hospitals. DBD NTP is an attractive, environmentally friendly solution for disinfection of moisture- and temperature-sensitive materials without the need of additional gases or chemicals and should be further explored. This is a proof-of-concept study, and the device can be scaled up for large capacity disinfection. This technology has the potential to prevent hospital-acquired infections, supply shortages, and reduce the waste produced by healthcare facilities. We envision that NTP devices based on this concept could also be adopted into other market sectors, such as ambulatory medicine, elderly homes, hospitality, and schools.

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Notes
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