



Disinfection of human coronavirus-229E on hospital cotton fabric via multi-cylinder dielectric barrier discharge plasma-generated reactive nitrogen species

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ABSTRACT

Viruses are a substantial burden on human health and the environment. Viruses persist based on the hydrophilicity and hydrophobicity of the materials they infect. Thus, preventing virus-contaminated materials from transmitting viruses is a significant challenge, especially in hospitals. The chemical disinfectants (e.g., NaOCl, ethanol, etc.) used in hospital settings bear numerous drawbacks. In addition, various medical devices and personal protective equipment (PPEs) are not adequately disinfected and reused, causing a shortage of devices and increasing cotton wastage. The persistence/inactivation of a virus depends on the nature of the materials and its interaction with those materials. In this study, we tested nonthermal plasma (NTP), particularly multi-cylinder dielectric barrier discharge (MCDBD) plasma-generated nitric oxide (NO_x), and found that it effectively inactivated human coronavirus 229E (HCoV-229E). Three different modes were used to evaluate the inactivation of HCoV-229E: i) direct plasma gas exposure, ii) plasma-activated water (PAW), and iii) virus-contaminated cotton fabric immersed in PAW. Our experimental results showed that direct plasma gas exposure and PAW decreased the virus titer (3.5–4 TCID₅₀/mL) and diminished the expression of the viral membrane and envelope proteins. We also examined the spike glycoprotein intensity in MRC-5 cells via fluorescence-activated cell sorting (FACS) and immunofluorescence analyses. The results of this study demonstrated that our MCDBD method can efficiently and cost-effectively disinfect hospital PPEs.

1. Introduction

The coronavirus pandemic has caused enormous damage worldwide. The virus spreads from an infected person to another person/environment in two ways: i) direct transmission through aerosolized droplets produced by sneezing and coughing and ii) virus-contaminated materials. The surface of a contaminated material contains viruses that can spread in multiple ways, and infected individuals may self-inoculate into the surrounding environment. Human coronavirus-229E, SARS-CoV-1, MERS, and SARS-CoV-2 persist differently on solid materials depending on various material characteristics such as hydrophilicity, hydrophobicity, porosity, wettability, absorption, liquid evaporation, isoelectric point, and temperature. The spread of the virus is correlated with the

material's porosity (e.g., paper, cotton fabric, wood, and KF94 mask) and nonporosity (e.g., stainless steel, plastic, cover glass, and copper). Several studies have indicated that viruses persist longer on nonporous surfaces than porous surfaces, and can persist for up to hr to nine days on nonporous surfaces at about 30 °C [1,2]. Previous studies have used HCoV-229E as a surrogate for SARS-CoV-2, which has similar persistence and inactivation mechanisms [3,4]. Therefore, we also chose HCoV-229E as a surrogate virus for SARS-CoV-2. The HCoV-229E and SARS-CoV-2 viruses both contain diverse or limited surface protein relationships that determine their persistence and inactivation. In addition, HCoV-229E is easy to manage and shares a close transformation history and significant physicochemical characteristics with extremely pathogenic coronaviruses that have previously emerged, such as MERS,

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SARS-1, and SARS-2. The duration over which coronaviruses persist on surfaces depends on the type of material. For example, Van Doremalen *et al.* [5] reported that SARS-CoV-2 persisted for up to 72 h on plastic and stainless steel surfaces and up to 4 h on copper.

Virus persistence primarily depends on the surface characteristics of the material, as liquid droplets persist on porous materials for shorter amounts of time (low contact angle) and on nonporous materials for longer amounts of time (high contact angle). Owen *et al.* [6] reviewed the stability of SARS-CoV-2 on porous surfaces. Hydrophilic materials have a higher surface area, and hydrophobic materials have a lower surface area. High roughness on the surface gives a large space for a virus to attach on materials and dry liquids more efficiently. Hence, this situation causes viruses are more inactivated on hydrophilic materials than on hydrophobic materials. At present, pathogen inactivation in materials, especially personal protective equipment (PPEs), is very important in hospitals. Preventing virus persistence or infections in hospitals and other healthcare settings is essential. In hospitals, viruses can spread from infected persons to bed sheets, aprons/gowns, duvets, clothes, and PPEs [7]. Generally, virus inactivation in fabrics is significant, and standard workplace fabrics possess comparable inactivation properties. Identifying what causes the virus to inactivate is crucial. For this reason, several disinfectants are used to deactivate coronavirus/pathogens and decontaminate the surfaces of hospital PPEs and community care centers. Certain disinfectants are effective for disinfecting surfaces against enveloped viruses in hospitals, particularly 70 % ethyl alcohol and 0.5–1 % sodium hypochlorite (corresponding to 5000 ppm) [8]. Other chemicals, such as sodium hypochlorite, povidone-iodine, formaldehyde, and glutaraldehyde agents, are considered practical tools against coronaviruses [9]. High concentrations of disinfectants cause immense impacts on environments containing microbiomes, animals, and humans, consequently accelerating antimicrobial resistance [10]. Specifically, chlorine reacts with dissolved organic matter in water and produces disinfecting byproducts (e.g., trihalomethanes, haloacetic acids), and these byproducts control microbial growth.

Chlorine is used to control microorganisms, especially bacteria and viruses; accordingly, it is used to disinfect virus-contaminated cotton fabric used in hospitals. Chlorine added to tap water exist together in free and combined forms. Free chlorine, including HOCl, OCl[−], and Cl[−] species, forms in tap water. Additionally, hypochlorous acid is unbalanced and can separate into hypochlorite ions. The chemical reactions are as follows: Eq. (1), 2).



High chloride concentrations promote a toxic effect on the environment and can damage cotton fabric surfaces. Thus, the concentration of sodium hypochlorite that inactivates the virus without affecting the nature of the environment and cotton fabric must be optimized. Well-established methods are required to control the virus without causing any side effects. The various methods used for inactivating coronaviruses by various methods, which are vaccination, X-rays, gamma radiation, phototherapy, ultraviolet radiation, and nonthermal plasma-generated reactive oxygen and nitrogen species (RONS) [11,12,13]. Ultraviolet radiation, gamma radiation and phototherapy are controlling the virus but not good for human health. If these are used for a long time alter the solid materials nature and also consume the high energy. For virus inactivation should be used other methods like NTP. Various plasma devices (plasma jet, corona discharge plasma, gliding arc discharge plasma, and dielectric barrier discharge plasma) control the viruses. All the plasma-generated RONS control the virus, but all devices produce less volume of PAW and consume more energy. MCDBD plasma effectively controls the virus; moreover, it produces a high volume of PAW and consume less energy.

Decontaminating assets requires a universal technique for creating

nonthermal plasma (NTP), particularly dielectric barrier discharge (DBD) plasma; accordingly, these plasmas have been broadly investigated and utilized in various fields such as environment, energy, and surface treatment [14]. Choi *et al.* [15] used plasma to generate RONS at electron temperatures of 0.8–3 eV and plasma densities of 3 to $5 \times 10^{12-16} \text{ cm}^{-3}$, and they hypothesized that these RONS could be used in bioscience and medicine.

The resulting NTP had advantageous decontamination properties. The assets featured several beneficial characteristics; for example, they were easy to turn on and off, required less maintenance, worked at ambient temperature with dry conditions, and were easy to handle. These types of features are important in virus inactivation. When using plasma-generated RONS, virus inactivation depends on the nature of the materials (e.g., porosity). Various kinds of plasma devices, such as jet plasma, nanosecond discharge plasma, micro-DBD, and surface-DBD, have been used to inactivate multiple types of pathogenic microorganisms, especially coronaviruses [2,16]. Sahun *et al.* [17] used a microsecond-pulsed DBD plasma system to inactivate the Wuhan and Omicron variants of SARS-CoV-2 on the surfaces of plastic and porous hospital materials (pillowcase and gown). On the plastic, the SARS-CoV-2 was reduced by 4.9 titers in TCID₅₀/mL; in the porous materials, it was reduced by 5.8 titers in TCID₅₀/mL for the porcine respiratory coronavirus.

Different reactive species, particularly ozone and nitric oxide, are required to inactivate viruses. PPEs contaminated with SARS-CoV-2 have been inactivated via ozone treatment [18,19]. Ozone concentrations of 10–20 ppm have been used for rapid virus disinfection [20]. Higher ozone concentrations used in shorter periods may result in more virus inactivations; however, high ozone concentrations can damage cotton cloth and the surrounding environment, and using lower concentrations may be desirable in certain situations. The Korean Ministry of Environment and the American Conference of Governmental Industrial Hygienists (ACGIH) recommended using O₃ concentrations lower than 0.05 ppm and NO_x concentrations lower than 3 ppm [15]. Based on this report, NO_x is preferable to O₃ from a safety point of view. Uhm [21] reported on excited nitrogen molecules in the gaseous state reacting with reactive oxygen species (ROS) (O₂, O₃, O, OH₂) in water to form nitrate (NO₃[−]), nitrite (NO₂[−]), and peroxyxynitrite (ONOO[−]) via the following reactions (3) to (9). These RONS are proportional and directly related to the pH, conductivity, and viral efficacy of a disinfectant.



Various atmospheric nonthermal plasmas, especially multi-cylinder dielectric barrier discharge (MCDBD) plasma, have been used to inactivate pathogens. This plasma produces a large volume of plasma-activated water (PAW), which has inactivated the highest percentage of bacteria and viruses when disinfection methods based on direct plasma gas exposure and PAW have been used. Previously, our group reported on a microwave plasma that generated 1000 μM of NO-enriched plasma-activated water, which reduced the HCoV-229E virus log order titer by 2 and also reduced the spike protein and E, M, and N gene expression [22].

The degree to which viruses spread/persist/inactivate in the environment depends on the types of solid materials on which the viruses reside. Fig. 1(A) illustrates how viruses interact with materials, and Fig. 1(B)–(E) show the contact angles of droplets on glass and cotton, an

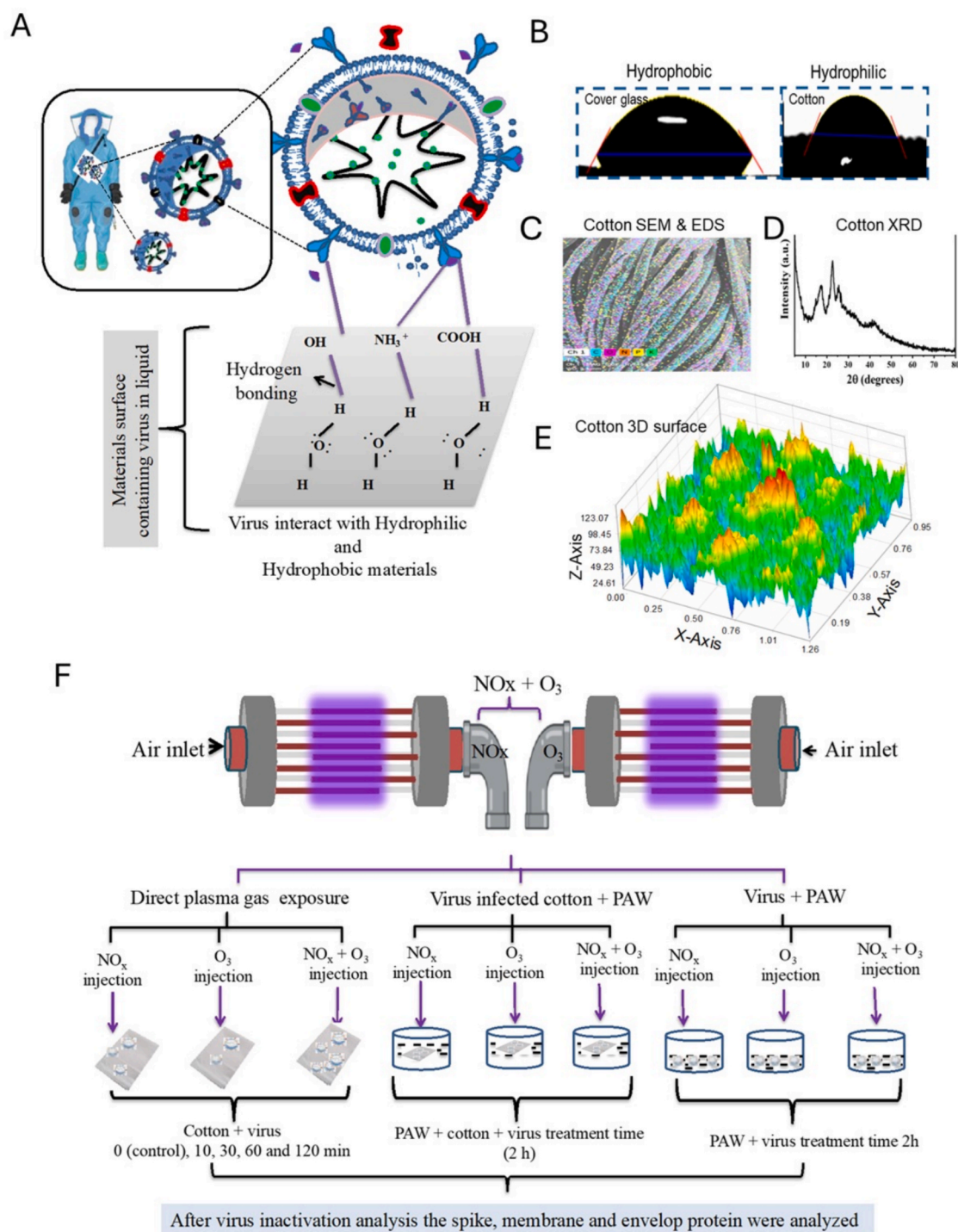


Fig. 1. Schematic diagram of the overall concept of virus persistence and inactivation. (A) Virus attached to PPEs, (B) contact angle of droplets on glass and cotton, (C) image of cotton generated using SEM with EDX, (D) XRD data for cotton, (E) 3-D surface topography of cotton, (F) different methods used in this study for inactivating HCoV-229E.

image of cotton obtained via scanning electron microscopy (SEM) with energy-dispersive X-ray spectroscopy (EDX), X-ray diffraction (XRD) data for cotton, and three-dimensional (3-D) surface topography of cotton, respectively. The spread of the virus is correlated with the material's characteristics particularly porosity. The materials (particularly PPEs) surface containing virus are inactivated at different methods particularly nonthermal plasma generated RONS, these RONS inactivate the viruses without causing any side effect on the materials and surrounding environment. In this study, we inactivated HCoV-229E with

RONS generated by MCDBD. We used three treatment methods to inactivate the virus on cotton fabric (as shown in Fig. 1F): 1) virus inactivation using direct plasma gas exposure, 2) virus inactivation on cotton fabric using PAW, and 3) virus inactivation using PAW. The rest of this paper is organized as follows. Section 2 describes the materials and methods, Section 3 presents the results, Section 4 discusses the significance of the results, and Section 5 summarizes the conclusions of the study.

2. Materials and methods

The [supplementary file](#) contains information on the experimental setup, including the purchased materials, virus titration, virus persistence, surface morphology of the solid materials, and contact angle. For virus inactivation, we used MCDBD plasma. As the plasma was being produced, we analyzed the current, voltage, optical emission spectrum (OES), and Fourier transform infrared (FTIR) spectroscopy of the gas. We used the alphacoronavirus HCoV-229E, a comparatively safe substitute for SARS-CoV-2. Different plasma exposures were used to inactivate the viruses. After virus inactivation, we analyzed the spike protein intensity and the expression of the membrane and envelope proteins.

2.1. Preparation of materials

Virus inactivation was performed using various porous and nonporous materials, including paper, cotton fabric, KF-94 masks, wood, stainless steel, plastic, cover glass, and copper. All the materials were cut into 1 cm² shapes, and then virus inactivation was performed. All the materials were washed with 75 % ethanol, cleaned twice with sterile distilled water, and UV sterilized for 15 min.

2.2. Statistical analysis

All tests were carried out using three independent sample replicates. The error bars represented the standard deviation from the mean values. Statistics were computed using Dunnett's/Tukey's various contrast analyses with two-way ANOVA via the PRISM software (version 8.0). The data were represented as follows: * indicated $p < 0.05$, ** indicated $p < 0.01$, and *** indicated $p < 0.001$.

3. Results

3.1. Virus persistence in long time intervals

We estimated the persistence (inactivation efficacy) of the HCoV-229E virus on the surfaces of various commonly used materials (e.g., paper, cotton fabric, wood, mask, stainless steel, plastic, cover glass, and copper). Fig. 2(A-H) show the virus persistence on the surfaces of the materials, expressed in units of 50 % of the tissue-culture infectious dose (TCID₅₀). During virus infection or inactivation process, we observed following phenomena. The cytopathic effect (CPE) induced in MRC-5 cells via viral infection indicates cell lysis or aggregated morphological variations in MRC-5 cells. However, the CPE is highly erratic, as it

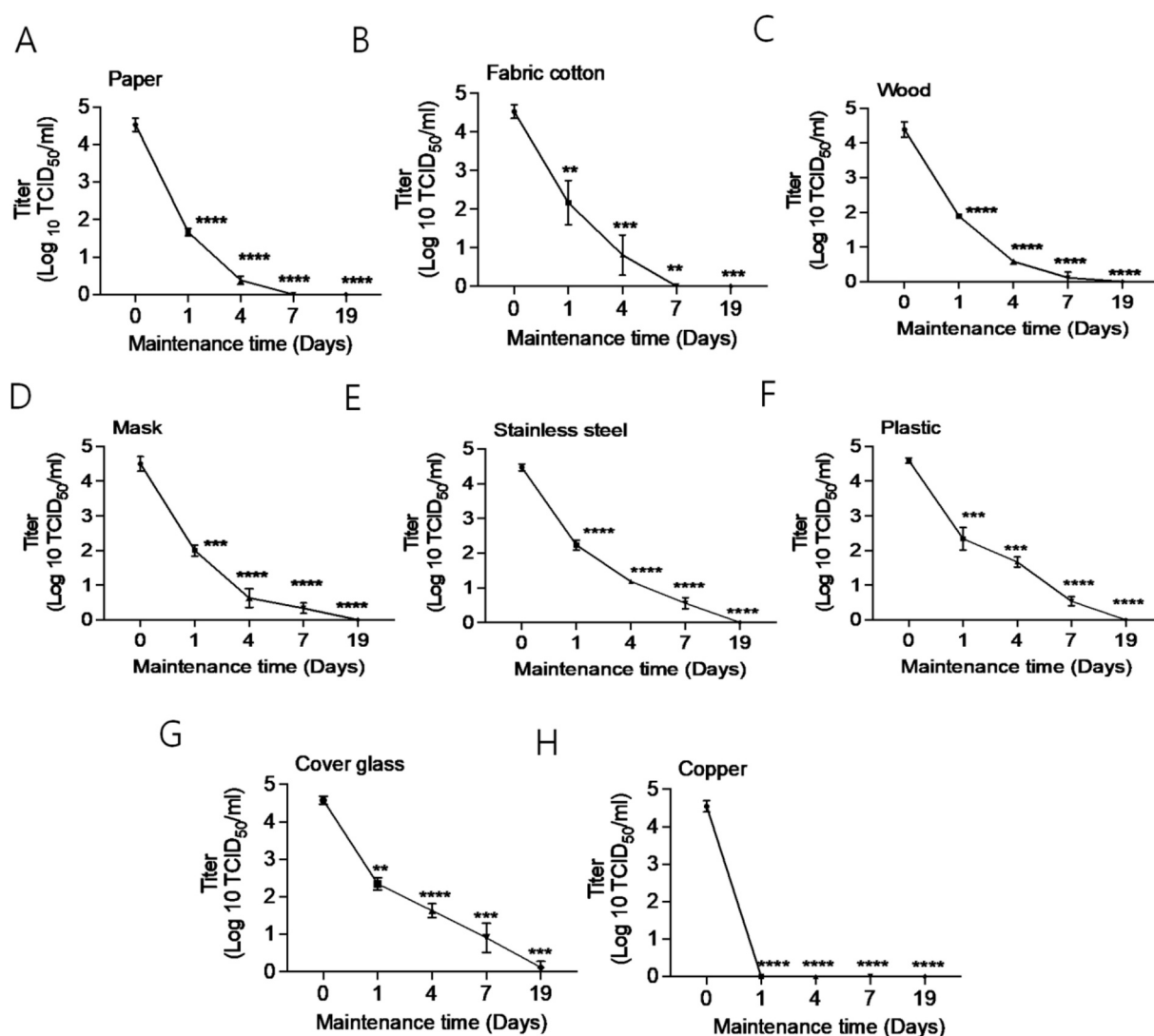


Fig. 2. Virus persistence over long time intervals. Virus-persistent titers are expressed in units of TCID₅₀/mL, and they were calculated using end-point titration on MRC-5 cells. The plots depict a decline in the logarithm of the virus titer over time. The data points and error bars correspond to the means and $\pm$ standard deviations, respectively.

depends on the cell number, viral titer, and maintenance time. We evaluated HCoV-229E infectivity in the MRC-5 cells every two days, and the CPE was checked up to once every two weeks (it was started on the fourth day). Viruses are persistent on surfaces for up to 19 days. The HCoV-229E virus in this study persisted on the solid surfaces of the cover glass, plastic, stainless steel, mask, wood, cotton fabric, and paper for 19, 7, 7, 7, 7, 4, and 4 days, respectively, and was nondetectable (ND) on the copper after one day. Accordingly, we estimated the persistence of HCoV-229E on the first eight materials. In the cover glass, plastic, stainless steel, mask, wood, cotton fabric, paper, and copper, the initial log order titer of the virus decreased from $4.39 \text{ TCID}_{50}/\text{mL}$ to 0.12 ± 0.13 , 0.54 ± 0.11 , 0.56 ± 0.13 , 0.34 ± 0.12 , 0.12 ± 0.13 , 0.81 ± 0.43 , $0.38 \pm 0.092 \text{ TCID}_{50}/\text{mL}$, and ND, respectively.

The virus persistence decreased with time. HCoV-229E was more constant on the cover glass, plastic, and stainless steel than on the mask, wood, cotton fabric, paper, and copper. No possible virus was detected on the copper after one day, while our previous study indicated that no viable virus was detected after 5 h [2]. Chin *et al.* [23] confirmed that SARS-CoV-2 persisted for two days in hydrophilic materials (wood and cloth), four days in glass and banknotes, and seven days in plastic and stainless steel. Wood is composed of tracheids and parenchymas fibers, and each fiber is composed of form cell walls that naturally produce porosity [24]. The cell wall has several layers, which are primarily made of cellulose (crystalline and amorphous). In addition, wood is a hygroscopic bio-based material that repeatedly interacts with the surrounding environment, which contains humidity [25,26]. Based on these conditions, the water content in the wood swells or shrinks as the water content increases or decreases, respectively [27], and the virus persistence level changes according to these conditions. Kasloff *et al.* [28], described the persistence of SARS-CoV-2 on porous materials, and found that viruses can persist for up to four days in stainless steel and plastic and one day in wood and clothes. Virus inactivation in materials is essential because if the virus persists for a long time, it can spread to other areas. Virus persistence and inactivation in materials are complex processes that depend on the environmental conditions and types of the materials. The characterization of the materials, including SEM with EDX, 3-D surface morphology, and X-ray diffraction (XRD), is described in here and the [Supplementary file](#) also.

3.2. SEM with EDX, 3-D surface morphology and X-ray diffraction (XRD)

SEM and EDX were used to provide high-resolution surface morphology and identify specific elements in the hydrophilic (paper, cotton fabric, wood, and mask) and hydrophobic (stainless steel, plastic, cover glass, and copper) materials. The surface morphologies of the various materials were analyzed at a magnification of 1000x. The [supplementary Figs. S1\(A\)-\(D\)](#) show the porous surface of the hydrophilic materials. The surfaces of the hydrophobic materials appeared to be relatively smooth (nonporous), as shown in [Supplementary Figs. S1\(E\)-\(H\)](#). The 3-D surface morphologies of the eight materials (paper, cotton fabric, wood, mask, stainless steel, plastic, glass, and copper) were analyzed, as shown in [supplementary Fig. S2](#). The 3-D images of the surfaces of the eight different materials show the textures of cavities and asperities, which had regular and irregular shapes. The 3-D images showed the surface topography and fiber alignment of the scaffold in the scanning range of 25–161 mm for the hydrophilic materials and 0.88–33.08 mm for hydrophobic materials. In [Fig. S2](#), red and blue represent the highest and lowest peaks and valleys, respectively. The surfaces of the hydrophilic and hydrophobic materials were rough and smooth, respectively. The surface position and thickness of the layers in materials are the key aspects that influence the surface quality, virus persistence, and inactivation. A 3-D analysis can provide a material's average surface roughness (Ra). However, Ra cannot provide information on the peak shape and wavelength, which means that Ra cannot distinguish between peaks and valleys. In contrast, the root-mean-

square roughness (Rq) is more sensitive to detecting deep valleys or high peaks in materials (as shown in [Table 1](#)). Based on these characteristics, the hydrophilicity or hydrophobicity of the materials were determined. Virus persistence/inactivation depends on the chemical composition and surface morphology of the material. The crystallinity (various diffraction angles 2θ) of the different materials was analyzed using XRD, the result of which are shown in [Fig. 3\(C\)](#).

3.3. Contact angle

We observed that the contact angle/evaporation of a Dulbecco's Modified Eagle Medium (DMEM) droplet gradually decreased over time, as shown in [Fig. 3\(A\)](#). The 10-min contact angles of the hydrophilic materials (paper, cotton fabric, wood, and mask) were 0.00° , 21.10° , 22.58° , and 68.08° , respectively, and the 30-min contact angles of the hydrophobic materials (plastic, stainless steel, cover glass, and copper) were 20.22° , 0.00° , 9.00° , and 0.00° , respectively. After 30 min, the droplet's total evaporation (contact angle) on each material was zero, except for the cover glass and plastic. The evaporation of microdroplets in hydrophilic and hydrophobic materials is a common phenomenon that is inter-correlated with virus persistence. In addition, the virus persistence and contact angle were correlated, as the liquid on the surface of the hydrophilic materials (paper, cotton fabric, wood, and masks) had a low contact angle ($>90^\circ$) and the liquid on the surface of the hydrophobic materials (cover glass, plastic, and stainless steel) had a high contact angle ($<90^\circ$). Hydrophilic materials such as paper, masks, and wood are easily interacted through hydrogen bonds with viral surfaces, and their contact angle (θ) is less than 90° . Hydrophobic materials (cover glass and plastic) contact angle (θ) $> 90^\circ$ nonpolar materials with a low affinity for water which repels water. In the environment, viral inactivation is involved by electrostatic interactions such as isoelectric point (pI), pH, ionic strength, hydrophobic effects, and small-scale non-covalent bonds (e.g., van der Waals forces). Virus proteins contain carboxyl and amine groups, these proteins have strong binding with hydrophilic materials and weak binding in hydrophobic materials this nature involves virus inactivation. [Fig. 3\(B\)](#) shows the droplet absorption and evaporation in porous and nonporous materials contaminated with viruses. Chatterjee *et al.* [29] reported that lower virus titers corresponded to higher temperatures and lower water droplets. They vaporized droplets containing coronaviruses at two different temperatures (25°C and 40°C). At 25°C , the tiny range of droplets evaporated after 6 s, and the large droplets evaporated after 27 s. At 40°C , the droplets evaporated within a second.

The droplet contact angle on the nonporous materials (stainless steel, plastic, cover glass, and copper) was visible for over 20 min. In contrast, the droplet spread constantly over the porous materials (paper, cotton fabric, mask, and wood), and in less than 20 min, the contact angle was almost invisible (i.e., the droplet becomes hidden inside the fibrous structure of the surface). The extent to which the virus stuck to the surface of the material was determined by the roughness/smoothness or the degree of hydrophobicity of the deposition. Liquid droplets are easily absorbed in porous surfaces within a shorter time interval compared to

Table 1

Surface roughness measurements (Ra and Rq) of the hydrophilic and hydrophobic materials.

Materials	Average surface roughness (Ra)	Root-mean-square roughness (Rq)
Paper	16.18	19.40
Cottonfabric	15.0	18.54
Wood	9.45	11.48
Mask	10.84	15.24
Stainless steel	0.08	0.10
Plastic	0.07	0.09
Cover glass	0.02	0.03
Copper	0.13	0.15

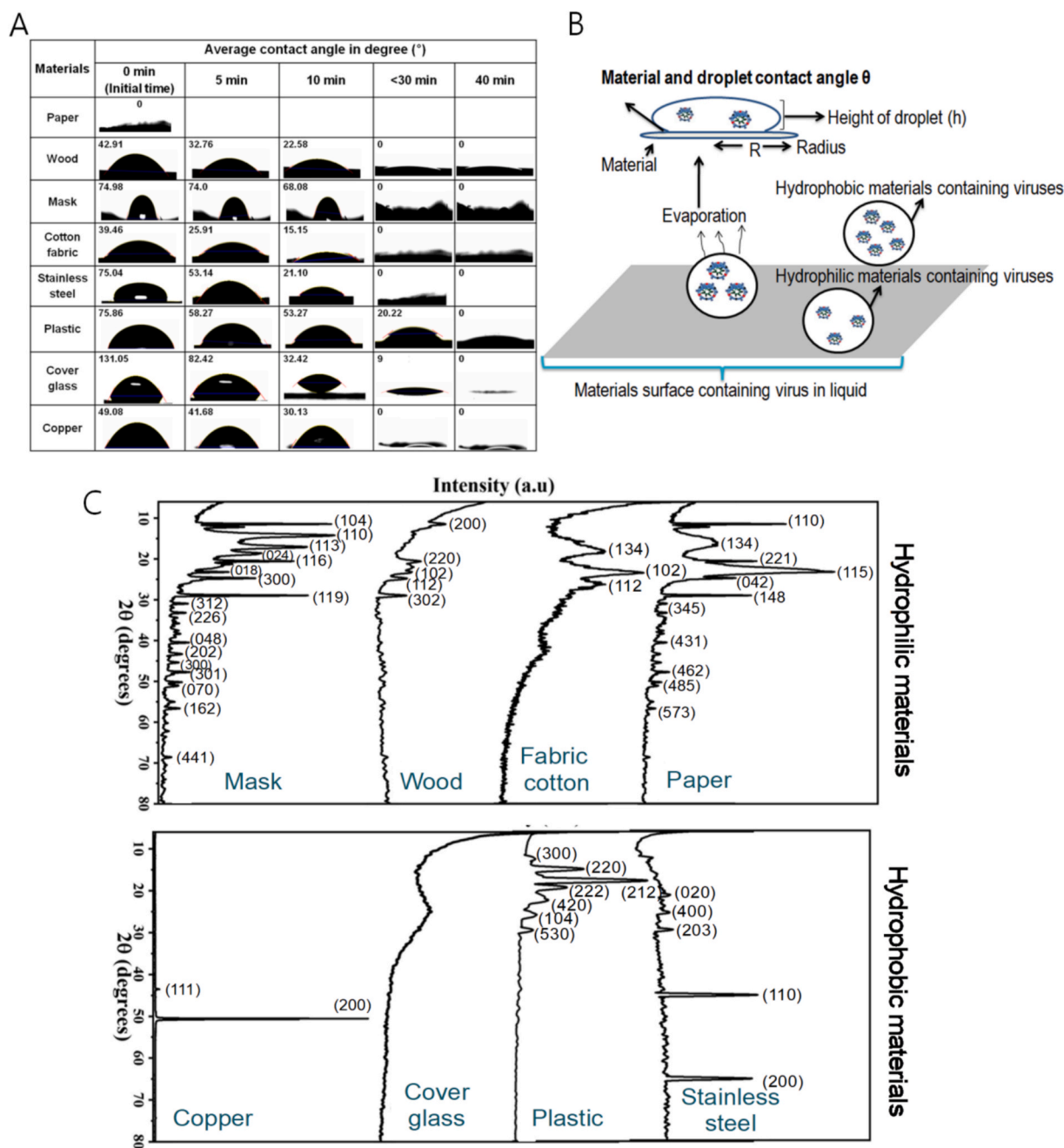


Fig. 3. Characterization of the eight materials: (A) contact angles for the different materials, (B) contact angles of the virus on the surfaces of the materials, (C) X-ray diffraction patterns of the hydrophilic and hydrophobic materials.

nonporous materials, and the size of the water droplets and contact angle decrease more rapidly, as shown in Fig. 3(A)-(B). Hydrophilic materials contain many diffraction peak intensities compared to hydrophobic materials, as shown in Fig. 3(C).

The degree to which viruses bonded to the surface of the materials depended on the softness or hardness and the hydrophobicity of the deposit. The surfaces of the porous materials adsorbed liquid droplets over time, reducing the droplet size and evaporating it more quickly than the nonporous materials. Chatterjee *et al.* [29] reported that the adhesion of surface-spreading droplets and contact angles was almost zero on porous materials, while droplets containing a thin film exhibited low evaporation, allowing viruses to persist for days. Virus adhesion to materials depends on various characteristics, including the pH, hydrophobicity/hydrophilicity, viral surface charge, and isoelectric properties of viral surface proteins [30].

In the hydrophilic materials, virus-containing droplets were absorbed into the materials, decreasing the moisture content (decreasing the contact angle) and rapidly drying the liquids. These processes reduced the virus titer. In addition, the moisture content of the hydrophilic materials initially bolstered the virus persistence, and increasing the maintenance time on the surface reduced the moisture content and virus titer. The hydrophobic materials retained their moisture levels for longer durations compared to the hydrophilic materials, and these conditions promoted virus persistence over a longer period. The virus was inactivated by the MCDBD plasma-generated reactive nitrogen species (RNS), as shown in Fig. 4(A).

3.4. Current and voltage

A root-mean-square (RMS) voltage of 6.7 kV generated by the

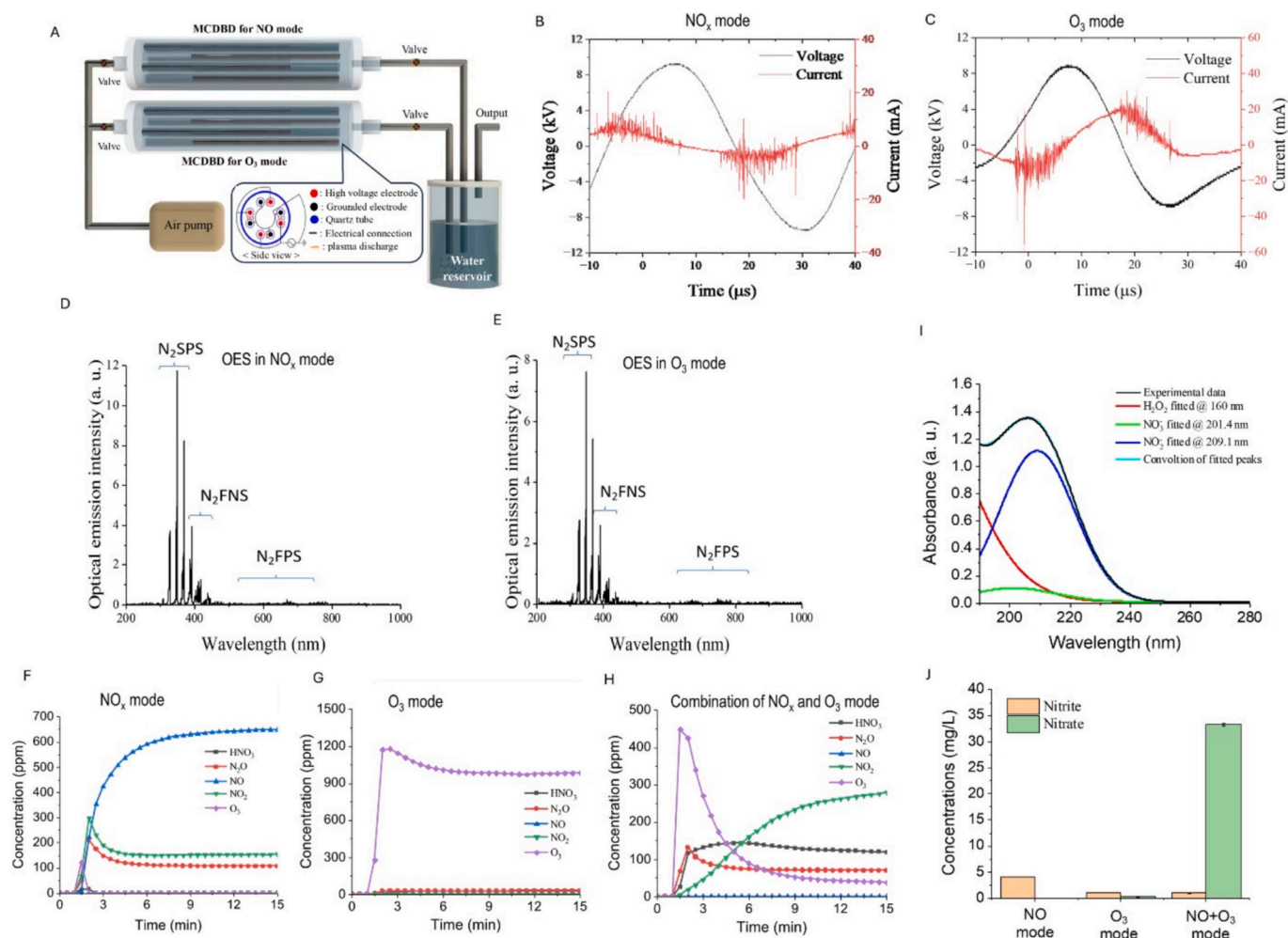


Fig. 4. Physical characteristics of the MCDBD: (A) schematic of the MCDBD and experimental setup, (B) voltage and current of the MCDBD in the NO_x mode versus time, (C) voltage and current of the MCDBD in the O₃ mode versus time with a duty ratio of 11.7 %, (D) OES in the NO_x mode, (E) OES in the O₃ mode, (F) gas FTIR in the NO_x mode, (G) gas FTIR in the O₃ mode, (H) gas FTIR in the combined NO_x and O₃ mode, (I) UV absorbance CD spectrum, (J) CD spectrum of the PAW for the NO₂⁻ concentrations of 4.05 mg/L, 1 mg/L, and 0.99 mg/L in the NO_x, O₃, and combined NO_x and O₃ modes, respectively, and for the NO₃⁻ concentrations of undetectable, 0.304 mg/L, and 33.26 mg/L in the NO_x, O₃, and combined NO_x and O₃ modes, respectively.

inverter using a continuous 20-kHz sinusoidal wave was used to produce the NO_x plasma, as shown in Fig. 4(B). The O₃ mode was used with an applied RMS voltage of 8.2 kV at 20 kHz with a duty cycle of 11.7 % (on time: 60 ms, off time: 450 ms) [31]. The measured voltage and current as a function of time and the duty cycle of the O₃ mode are shown in Fig. 4 (C). The applied duty cycle was determined using Eq (10) and the electrical power (*P*) was measured according to the voltage versus current curve via Eq (11). Where *T* is the period of the voltage curve. The electrical power of the NO_x and O₃ modes were 2.6 W and 1.2 W, respectively.

$$\text{Duty ratio}(\%) = \frac{\text{ontime}}{\text{ontime} + \text{offtime}} \times 100 \quad (10)$$

$$P = \frac{1}{T} \int_0^T v(t) \times i(t) dt \text{ [W]} \quad (11)$$

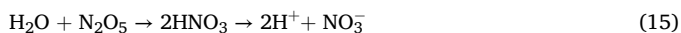
3.5. Optical emission spectrum (OES), gas-FTIR, and CD spectrometer

The OES of the MCDBD is shown in Fig. 4(D)-(E), which show that the measured spectra of the NO_x and O₃ were similar. Both spectra have similar nitrogen bands showing an N₂ second positive structure (SPS), N₂ first negative structure (FNS), and N₂ first positive structure (FPS) at 300–400 nm, 400–500 nm, and 550–800 nm, respectively. MCDBD-

generated reactive oxygen and nitrogen species were measured via gas FTIR (Matrix-MG5, Bruker) analysis, which was performed using three modes (NO_x, O₃, and a combination of NO_x and O₃ modes) during virus inactivation using direct plasma gas exposure, as shown in Fig. 4(F)-(H). In the case of the NO_x mode, the NO_x was mainly produced at 630 ppm. In addition, the NO₂ and N₂O were produced at 150 and 105 ppm, respectively. In the O₃ mode, the O₃ was produced primarily at 980 ppm, and the N₂O and HNO₃ were produced at 25 ppm. Other than this, no other chemical compounds were produced, and the O₃ was observed within 1 min. Combining the NO_x and O₃ modes increased the diversity of the chemical species. As the plasma was being generated, the O₃ production rapidly increased and decreased after 30 s. The HNO₃ and N₂O were produced at 120 and 70 ppm, respectively, and even though the other chemical compounds were less prevalent, the NO₂ concentration was maintained at 260 ppm for up to 15 min.

After a three-hour plasma treatment, the PAW was checked for the RONS using a UV absorbance circular dichroism (CD) spectrometer (J-815, JASCO, Easton, MD 21601, USA) with a wavelength range from 190 to 280 nm, as shown in Fig. 4(I)-(J), and an absorbance spectrum resolution of 0.5 nm. The RONS (H₂O₂, NO₂⁻, and NO₃⁻) concentrations in the PAW were measured using UV absorbance spectra via the Beer-Lambert law [32,33,34]. The H₂O₂, NO₃⁻, and NO₂⁻ absorbances in the PAW were deconvoluted using Gaussian functions with centers of 160

nm, 201.4 nm, and 209.1 nm, respectively. These H_2O_2 , NO_3^- , and NO_2^- concentrations were calculated using the NaNO_2 and NaNO_3 calibration table [34]. According to the experimental results in Fig. 4(J), The concentration of nitrate in the combination of $\text{NO} + \text{O}_3$ mode has been higher than PAW- NO_x and PAW- O_3 . It indicates that nitrate formation was enhanced by reactions between the NO and O_3 . Following reaction (Eq.12–15) in happened, consequently, $\text{NO} + \text{O}_3$ mode accelerates HNO_3 formation for a precursor of NO_3^- in PAW as shown in Fig. 4(H–J).



3.6. Inactivation of viruses on cotton fabric using plasma gas exposure

The MCDBD plasma produced free electrons with higher energy, ultraviolet (UV) radiation, and various RONS generated in the four pairs of electrode gaps [35]. The MCDBD plasma gas generated NO_x and O_3 , which inactivated HCoV-229E. This HCoV-229E inactivation assay was tested in MRC-5 cells using five different conditions: 1) negative control (MRC-5 cell only), 2) positive control (virus on cotton fabric without plasma gas exposure), 3) NO_x gas exposure (virus on cotton fabric plus NO_x plasma gas), 4) O_3 gas exposure (virus on cotton fabric plus O_3 gas) and 5) a combination of NO_x and O_3 gas exposure (virus on cotton fabric plus NO_x and O_3 plasma gas). After different treatment timings (0, 10, 30, 60, and 120 min), the virus was inoculated into the host cell, and the CPE was observed for up to 14 days. The negative control did not exhibit any CPE, and in the positive control (without plasma gas exposure), the virus titer decreased as the plasma treatment time increased, as shown in Fig. 5(A). Initially (at 0 min), the virus titer was 4.42 TCID₅₀/mL, and after 2 h, it was 3.17 TCID₅₀/mL. The NO_x , O_3 , and combined NO_x and O_3 plasma gas exposure reduced the virus titer from 4.42 TCID₅₀/mL to 1.39, 2.23, and 1.83 TCID₅₀/mL, respectively, after 2 h, as shown in

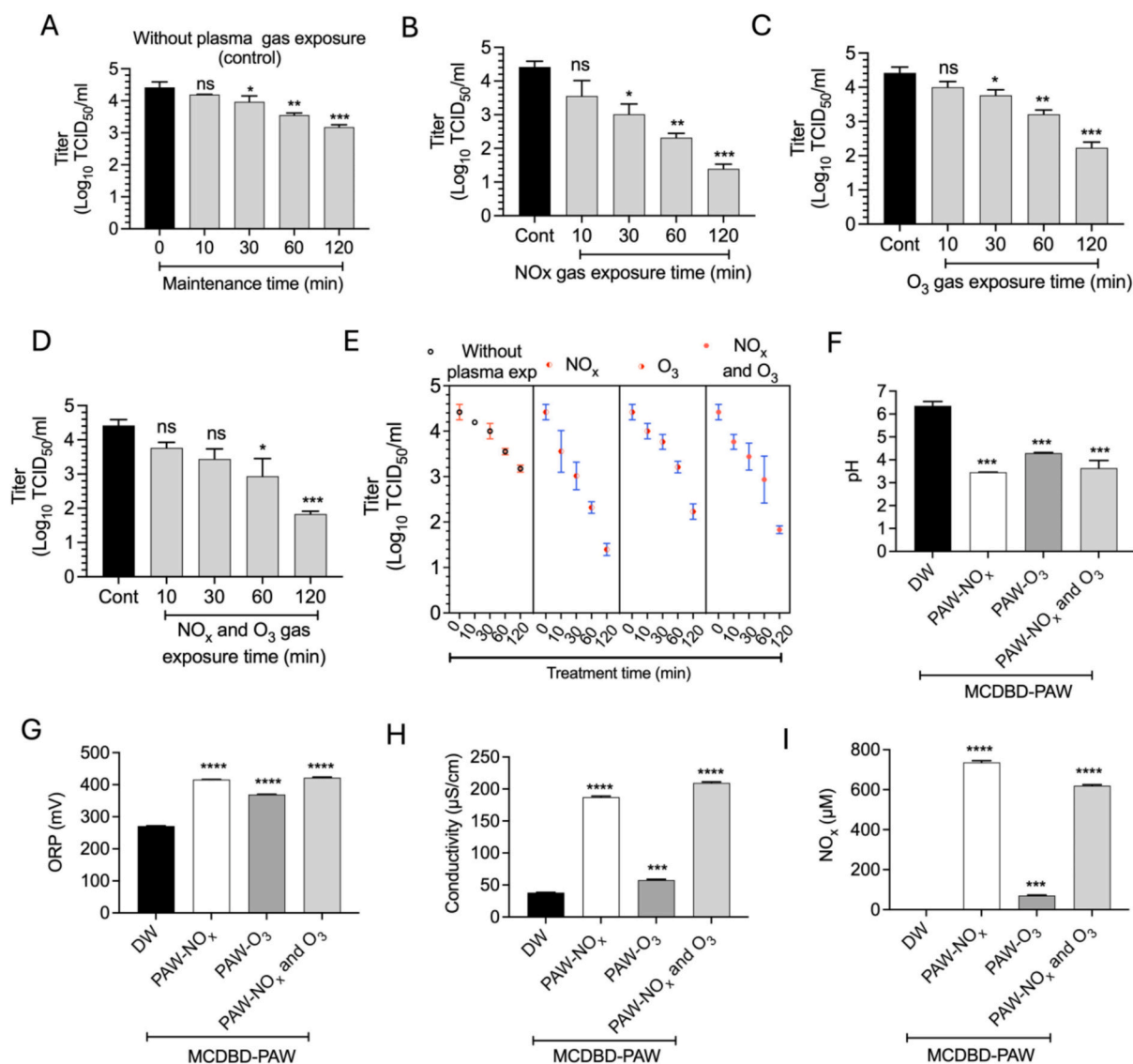


Fig. 5. Virus inactivation after the plasma gas was exposed to water and physicochemical characteristics of the PAW. (A) Virus inactivation without plasma gas exposure (control). Virus inactivation with plasma gas exposure for the (B) NO_x , (C) O_3 , and (D) combined NO_x and O_3 treatments. (E) Dependence of the virus persistence reduction on the plasma gas exposure and treatment time. Physicochemical characteristics of the MCDBD plasma-generated PAW in terms of the (F) pH, (G) ORP, (H) conductivity, and (I) NO_x concentration.

Fig. 5(B)–(D). The virus titer gradually decreased with time (from 0 to 120 min), and the extent to which the virus persistence decreased depended on whether or not it was exposed to the plasma gas, as shown in Fig. 5(E).

3.7. Physical-chemical characterization of NO_x-enhanced PAW

The plasma gas exposed to water significantly decreased the pH from 6.36 ± 0.19 to 3.46, 4.29, and 3.64 in the PAW-NO_x, PAW-O₃, and combined PAW-NO_x and O₃, respectively, as shown in Fig. 5(F). The oxidation–reduction potential (ORP) reflected the general oxidation capacity of the PAW. Before the plasma gas was injected into the water, the ORP was 217.0 ± 0.8 mV. In contrast, the ORP values of the PAW-NO_x, PAW-O₃, and combined PAW-NO_x and O₃ significantly increased,

reaching 416.1, 369.5, and 421.7 mV, respectively, as shown in Fig. 5 (G). As shown in Fig. 5(H), the conductivity of the water was 37.93 ± 0.52 μ S/cm before the treatment; after the treatment, the conductivity increased to 57.60–209.52 μ S/cm. Fig. 5(I) shows the concentration of the PAW-NO_x after the three different treatments. The PAW-NO_x treatment reached a NO_x concentration of 736.34 ± 8.73 μ M, and the PAW-O₃ and combined PAW-NO_x and O₃ treatments reached 70.16 and 619.42 μ M, respectively. While the plasma gas was exposed to water, the NO_x concentration in the water increased (NO₂ and NO₃ further converted into HNO₂ and HNO₃, respectively), particularly in the PAW-NO_x injection. The water injected with O₃ gas had 70.16 μ M of NO_x. Lim *et al.* [36] have demonstrated that the amount of O₃ generated (gas phase) by dielectric barrier discharge plasma (DBDP) depends on the temperature variations within the duty cycle. They found that O₃ was produced at

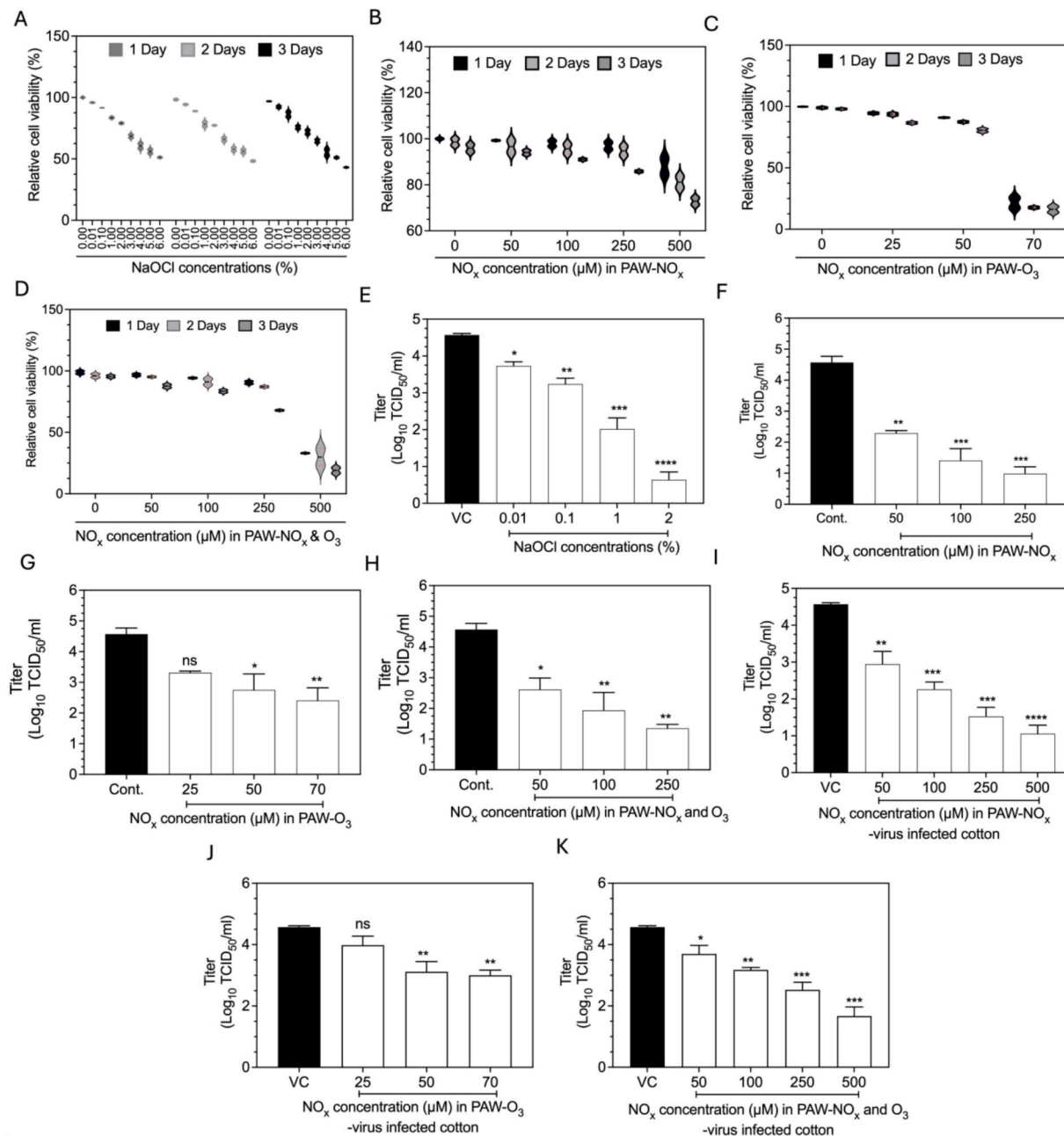


Fig. 6. Relative *in-vitro* cell viabilities in MRC-5 cells and inactivation of HCoV-229E using different plasma-generated NO_x concentrations. Cell viability for the (A) NaOCl, (B) PAW-NO_x, (C) PAW-O₃, and (D) combined PAW-NO_x and O₃ concentrations. HCoV-229E inactivation in NaOCl and PAW for the (E) NaOCl, (F) PAW-NO_x, (G) PAW-O₃, and (H) combined PAW-NO_x and O₃ concentrations. Inactivation of contaminated HCoV-229E on cotton fabric using the (I) PAW-NO_x, (J) PAW-O₃, and (K) combined PAW-NO_x and O₃. Note: VC (as well as "Cont.") stands for "virus control" (i.e., the control group).

100 °C, then the amount of O₃ produced decreased as the temperature increased, and then N₂O₅ was produced as the temperature increased to 60 °C. This N₂O₅ reacted with H₂O and produced NO₃⁻. As a result of this process, the PAW-O₃ treatment produced NO_x. Based on this fact, the NO_x concentration decreased the pH and increased the conductivity and ORP. Plasma generated (PAW) H₂O₂ is lower the pH and NO₂⁻, NO₃⁻ increase the EC, and during acidic conditions higher the ORP in PAW. These parameters synergistically enhance viral inactivation.

3.8. Virus inactivation in hospital cotton fabric

When we used MCDBD plasma to treat the cotton fabric contaminated by HCoV-229E, we used direct plasma gas exposure and PAW. Three kinds of plasma generation modes involving different gas injections were used to generate the PAW: i) NO_x injection, ii) O₃ injection, and iii) a combined NO_x and O₃ injection. These virus experiments were conducted in biosafety level 2 (BSL-2) facilities.

3.9. Toxicity analysis of PAW

To conduct a toxicity analysis, we used NaOCl and different concentrations of the plasma gases (NO_x, O₃, and the combination of NO_x and O₃). They were injected into distilled water to fabricate PAW. The *in-vitro* cytotoxicity of the MCDBD plasma-generated PAW-NO_x (0 to 500 μM of NO_x) at various concentrations of NO_x and NaOCl was assessed on MRC-5 lung fibroblast cells using an Alamar blue assay. The different concentrations of NO_x and NaOCl were added into the cells, and the cell viability was measured for up to seven days after the treatment. We observed a decrease in the relative cell viability as the NaOCl concentration and incubation time increased. At an NaOCl concentration of 6 %, 43.1 % of the cells survived up to seven days, and at an NaOCl concentration of 2 %, more than 70 % of the cells survived, as shown in Fig. 6(A). Different plasma-induced PAW cytotoxicities are shown in Fig. 6(B)-(D). The effect of different concentrations (0–500 μM of NO_x) of the PAW-NO_x resulted in a less pronounced cytotoxic effect. The relative cell viabilities of 88.0 %, 81.2 %, and 72.9 % occurred when the cells were incubated for one, two, and seven days, respectively, and the contact time had strong cytotoxic effects for 500 μM of NO_x. More than 85 % of the cells were viable in the NO_x concentration range of 50–250 μM. The PAW-O₃ induced the maximum cytotoxic effect (viability at 20 %) at a NO_x concentration of 70 μM, and 0–50 μM of NO_x had a less toxic effect (viability at 80 %). Other combinations of NO_x and O₃ plasma gas injected into the PAW induced a toxicity of 70 % for 500 μM of NO_x and a lower toxicity for 0–250 μM of NO_x. Before examining the efficacy of the virus inactivation, the cytotoxicity of the PAW-NO_x in MRC-5 cells was determined using an Alamar blue assay. Three modes that generated different concentrations of NO_x were used to conduct this analysis. The results indicated that 80 % of the MRC-5 cells survived in 250 μM of NO_x generated in two modes (NO_x and a combination of NO_x and O₃). This optimum concentration (250 μM of NO_x) suggested that a non-toxic environment was present in the cells; thus, these concentrations were used for further virus inactivation analyses of the PAW. Cytotoxicity analysis provides a potential measurement of host cell death (MRC-5) caused by PAW or solid materials. This happens mainly between the materials and PAW. If the materials produced toxic effects after the PAW treatment, the materials (cotton fabric) may promote the inflammation to the patient. Less or safe levels of PAW reduce the virus titer and also reduce the cotton fabric coming out, irrigation and tissue damage in the hospital environment. This cytotoxicity clearly showed that the PAW treatment is biocompatible for washing.

3.10. Virus inactivation in liquid and cotton fabric

The inactivation capabilities of different PAW mixtures for HCoV-229E viruses on cotton fabric were evaluated. Plasma-discharged water contains various concentrations of RNS, which are potential

oxidants that can inactivate the viral surface protein and nucleus. To generate PAW using MCDBD plasma, we mixed MCDBD plasma-generated RONS with water using air bubbles to form reactive species. PAW contains different RONS, particularly NO₂⁻, NO₃⁻, hydrogen peroxide (H₂O₂), ozone (O₃), and hydroxyl radicals (•OH). The HCoV-229E suspension was treated/incubated with different concentrations (0–500 μM) of plasma-activated NO_x. After 14 days of treatment, an analysis of the reduction in the virus persistence was conducted, and the results were expressed in units of TCID₅₀/mL. We used sodium hypochlorite (NaOCl) as a disinfectant to reduce the HCoV-229E contamination in water; 0.01 % of NaOCl reduced the virus titer to 0.84 TCID₅₀/mL, and 2 % of NaOCl reduced the virus titer from 4.57 to 0.64 TCID₅₀/mL. These results indicated a 99.99 % reduction in the virus persistence, as shown in Fig. 6(E). Increasing the NaOCl concentration effectively controlled the virus; however, NaOCl discharges contribute to environmental pollution. Therefore, to avoid environmental contamination, we used alternative methods to control HCoV-229E.

In Section 2, we explained the inactivation of HCoV-229E using PAW-NO_x generated by different plasma gas systems. In this study, we inactivated HCoV-229E in PAW and in virus-contaminated cotton fabric immersed in a different concentration of NO_x (from 0 to 500 μM). First, we investigated different concentrations of PAW-NO_x (0–250 μM) that inactivated HCoV-229E. A NO_x concentration of 250 μM reduced the virus titer from 4.57 to 0.98 TCID₅₀/mL; lower NO_x concentrations resulted in a smaller degree of virus inactivation. PAW-O₃ inactivated HCoV-229E at a NO_x concentration of 70 μM, which reduced the virus titer from 4.57 to 2.41 TCID₅₀/mL, and other NO_x concentrations resulted in less effective inactivation. However, achieving a higher degree of virus inactivation at lower NO_x concentrations is desirable; for this reason, we combined the NO_x and O₃ modes. This mode generated 250 μM of NO_x, reducing the virus titer from 4.57 to 1.34 TCID₅₀/mL. However, this mode did not attain a virus inactivation level greater than that of the NO_x mode. Between treatments, the NO_x generated by the NO_x mode (250 μM) reduced the viral titers by an amount larger than that of the NO_x generated by the combined NO_x and O₃ mode, as shown in Fig. 6(F)-(H).

To assess the inactivation of viruses contaminating the cotton fabric, 100 μL of the HCoV-229E virus was added to the surface of the fabric, maintained at room temperature for 5 min, and then immersed in different concentrations of NO_x obtained from different plasma modes. After 1 h of treatment, the PAW-NO_x with 500 μM of NO_x reduced the virus titer from 4.57 to 1.05 TCID₅₀/mL, the PAW-O₃ with 70 μM of NO_x reduced the virus titer from 4.57 to 3 TCID₅₀/mL, and the combined PAW-NO_x and O₃ with 500 μM of NO_x reduced the virus titer from 4.57 to 2.9 TCID₅₀/mL, as shown in Fig. 6(I)-(K). The virus inactivation primarily depended on the NO_x concentration. Materials' surface nature (chemical structures) is always changed based on environmental conditions, and PAW enhances the functionality of the materials, avoids surface deterioration, and increases the virus inactivation level. SEM images, the porous nature favors virus persistence (interaction between the materials and virus is weak), when compared with nonporous materials (interaction between the materials and virus is strong). All porous materials contain carbon group, this C interacts with water containing O and to form a C=O, these conditions may be involved in reducing the virus. In non-porous, they don't have these types of elements. In this study, we tested virus inactivation within 10 min and 5 h after making PAW. At the end of the study, the NO_x concentration and virus inactivation did not change significantly. In our previous report, microwave plasma generated NO_x concentrations were very stable at low temperatures (–80 °C) and decreased at room temperature when increasing the time (days) [22]. After virus inactivation was accomplished using the desired concentrations of NO_x, the virus protein expression was analyzed in MRC-5 cells.

3.11. Evaluation of virus protein expression in MRC-5 cells using immunofluorescence and flow cytometry after virus inactivation

After virus inactivation, we determined the viral spike protein expression in MRC-5 host cells. This assessment was carried out using three different PAW-NO_x concentrations to inactivate the viruses contaminating the cotton fabric. After the treatments were completed, the treated virus was added into MRC-5 cells. These cells were bound with viral spike proteins and DAPI (4',6-diamidino-2-phenylindole), and then visualized using confocal microscopy. Confocal images of the group treated with PAW-NO_x and the control group (i.e., with and without virus contaminations, respectively) were compared. The spike protein is a glycosylated surface spike protein, and these proteins interact as mediators of viruses entering the host cells. Fig. 7(A) illustrates the spike protein fluorescent intensity images of the HCoV-229E-infected MRC-5 cells treated with PAW-NO_x and of the control group. The DAPI binded to uninfected MRC-5 host cells, producing an enhanced blue color. Red fluorescent intensity indicated HCoV-229E infection in the MRC-5 cells, with a higher infection indicated by a higher intensity and a lower infection indicated by lower intensity. The PAW-NO_x treatment effectively reduced the expression of the viral spike glycoprotein in the MRC-5 cells compared to the virus control. Among the different treatments, the PAW-NO_x (NO_x mode) inactivated the virus spike protein more effectively than the PAW-O₃, combined PAW-NO_x and O₃, and control group. At the same time, the image of the control cells showed no viral infection.

Furthermore, after the plasma gas and PAW treatments, we quantified the expression of the virus spike protein particles in HCoV-229E-infected MRC-5 cells using flow cytometry analysis. Three methods were used in the flow cytometry analysis: direct plasma gas exposure (shown in Fig. 7(B)), PAW (shown in Fig. 7(C)), and virus-infected cotton fabric immersed in PAW (shown in Fig. 7(D)). In all the groups, similar fluorescence intensity patterns were exhibited. After the plasma treatment, the fluorescence intensity was lower than that of the control group; the NO_x mode for direct plasma gas exposure and PAW exhibited significantly lower intensities compared to those of the other treated groups (i.e., O₃, combination of NO_x and O₃).

3.12. Plasma-activated NO_x water downregulated by antiviral host response gene during HCoV-229E infections on cotton fabric

The human coronavirus (HCoV) contains four constrictive proteins: the spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins. All proteins involved in different activities, particularly those of viruses, enter the host cells through spike proteins [37]. The E protein is involved in virion assembly and morphogenesis, and the M protein is encapsulated in the viral membrane of structural proteins [38]. Overall, the results indicated that plasma treatment effectively inactivated the virus on the cotton materials. In contrast, the immunofluorescence and flow cytometry results showed that the plasma treatment significantly reduced the spike protein level. In addition to spike protein inactivation, E and M protein inactivation is also essential. Furthermore, analyzing molecular-related investigations is critical because virus persistence, propagation, and inactivation primarily involve mRNA. Friedman *et al.* [39] reported that approximately 319 genes are relevant for the antiviral response, and a selection of these genes are activated during HCoV-229E infection in MRC-5 cells. They used virus-infected MRC-5 host cells to analyze the antiviral host response genes, which were quantified using quantitative polymerase chain reaction (qPCR) analysis. To further add to our results, we also investigated whether PAW-NO_x could reduce the expression of the HCoV-229E virus at a molecular (mRNA) level. We found that the PAW-NO_x decreased the expression of M and E gene messenger RNA (mRNA) compared to the PAW-O₃, combined PAW-NO_x and O₃, and control group, as shown in Fig. 7(E)-(F).

4. Discussion

The human respiratory coronaviruses (HCoV-229E, SARS-CoV-2, MERS, and SARS-CoV-1, *etc.*) are persistent or inactivated on the surface of hydrophilic and hydrophobic materials. The persistence or inactivation of the viruses depend on the material's porosity, adsorption, evaporation, hydrophobicity, relative humidity, isoelectric point, temperature, pH, and other characteristics. Droplets containing viruses are spread into the environment via virus-contaminated materials. Viruses can adhere to the surface of the materials and persist for different durations, depending on the environmental conditions. Viruses can also persist on objects, and they can spread themselves to other locations through inhalation or contact. We evaluated the persistence of HCoV-229E in household and PPEs materials for up to 20 days, as shown in Fig. 2. The results showed dramatic differences between hydrophilic and hydrophobic materials. HCoV-229E persisted for longer on impermeable (hydrophobic/nonporous) surfaces than on porous surfaces, as shown in Fig. 2. Kampf *et al.* [1] also reported that viruses persist for longer time intervals on hydrophobic materials.

The porosity of the materials (both porous and nonporous) primarily determined whether the viruses persisted for short or long time intervals. Viruses were less persistent on the paper because of the high evaporation rate (<10 min), and the other solid materials required more than 30 min to evaporate the water on their surfaces (except for copper). In the porous materials, water droplets were adsorbed/evaporated quickly, which enhanced the virucidal efficacy. In the nonporous materials, water droplets were adsorbed/evaporated more slowly; as a result, the viruses persisted for a longer time interval, as shown in Fig. 3 (A). Droplet or fomite evaporation is associated with the persistence of viruses. Smaller contact angles (for porous materials) have shorter evaporation timescales. This implies that these droplets evaporate quickly (<10 min), thereby reducing the virus' persistence. The nonporous materials (except copper) contained droplets that required longer evaporation times (>10 min), and this increased the duration of the virus' persistence and the probability of viral transmission. During evaporation, the mole fraction of the solvent increased and attained a critical super-concentrated intensity, which generated precipitation, which subsequently increased the solute concentration (owing to continuous evaporation). When the evaporation accelerated, the liquid mass was entirely drained, and the evaporation stopped. The concentration of solutes increased their solubility limit as water evaporated from the solution, resulting in supersaturation. This effect caused the spontaneous formation of small clusters (nuclei) of particles in the solutions. These nuclei acted as primary sites for crystal formations. Perhaps this type of effect, combined with other parameters (e.g., temperature and relative humidity), is capable of inactivating more viruses [40].

A material with a porous surface possesses a larger surface area and a smaller diffusion path; these properties enabled a high degree of virus inactivation in the paper, cotton fabric, and wood, and also revealed that droplets containing viruses evaporate quickly. Bhardwaj and Agrawal [41] investigated the persistence of coronaviruses on different surfaces, such as glass, copper, polypropylene, and stainless steel. The evaporation/drying times for 5 μ L of water droplets on glass, polypropylene, and stainless steel were 84 h, 96 h, and 24 h, respectively. They discovered that thin film surfaces containing water droplets were correlated with the reduction in the virus titer and with the drying timescale of the droplets.

In the materials, the liquid droplets containing viruses were evaporated or adsorbed, and were correlated with the material's topography. Supplementary Figs. 1 and 2 show the SEM images and 3-D topographies of the materials. Supplementary figures S1(A)-(D) show the pores in the paper, cotton fabric, KF-94 mask, and wood, respectively. Supplementary figures S1(E)-(H) show the nonporous nature of the stainless steel, plastic, cover glass, and copper, respectively. Porous surfaces provide a larger surface area and a smaller dispersion, which assists the virus to

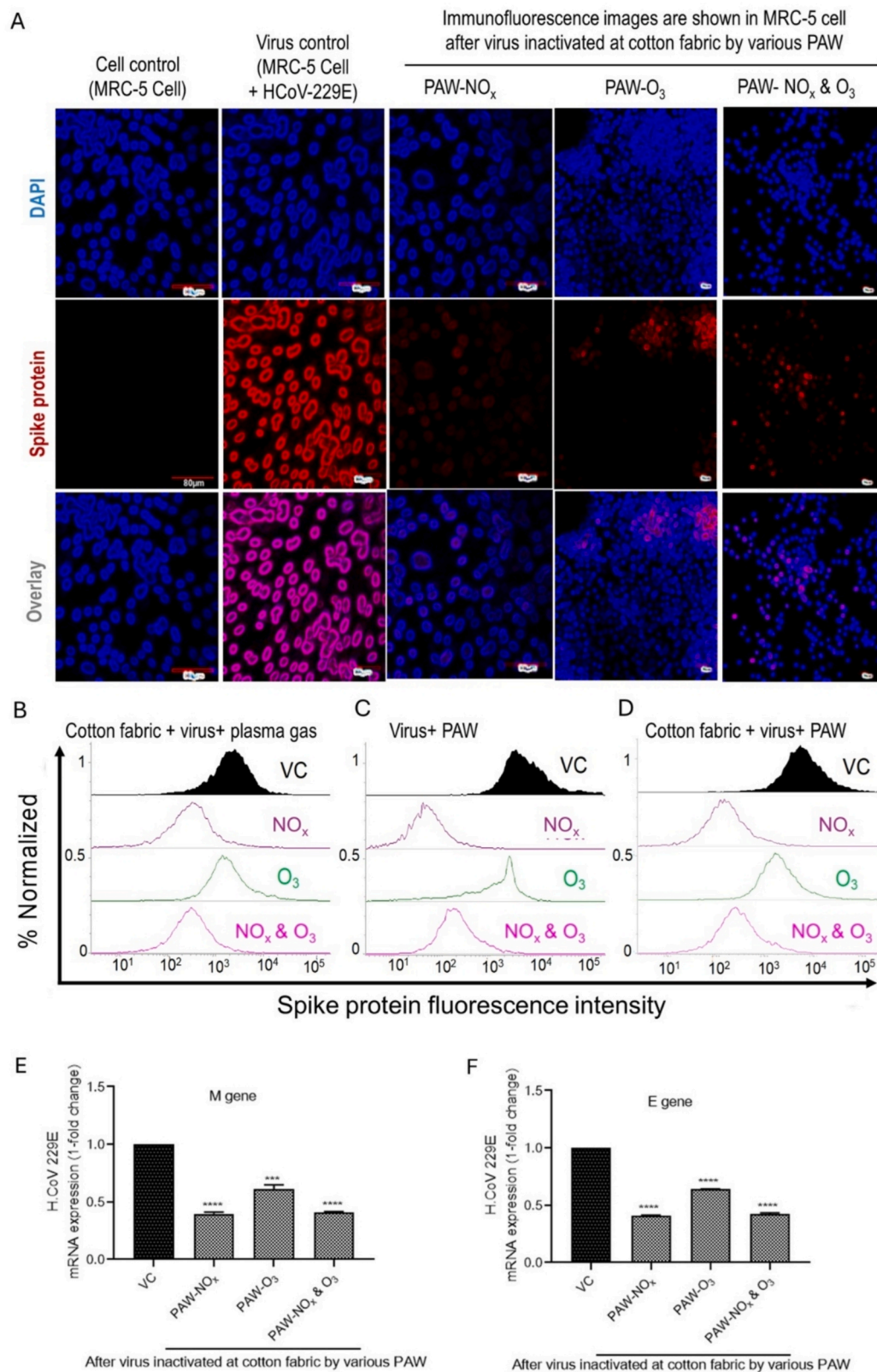


Fig. 7. Plasma treatment reduced the appearance of spike, membrane (M), and envelope (E) proteins. (A) Representative immunofluorescence images of MRC-5 cells infected with HCoV-229E (images of the results with and without plasma treatment are shown). Flow cytometry analysis of spike protein intensity of MRC-5 cells infected with HCoV-229E after plasma treatment: (B) direct plasma gas exposure, (C) PAW, and (D) virus-infected cotton fabric with PAW. Expression of viral protein: (E) M gene and (F) E gene. Note: VC stands for “virus control” (i.e., the control group).

contact materials, thus leading to virus inactivation. These conditions allowed the liquid droplets containing the virus to dry faster than those on the nonporous materials, and this naturally produced a significant level of virus persistence/inactivation. According to the 3-D topography, the R_a of the hydrophilic materials was above $9\text{ }\mu\text{m}$, and the R_q was above $11\text{ }\mu\text{m}$, as shown in Figs. S2(A)-(D). In contrast, the R_a and R_q of the hydrophobic materials were less than $1\text{ }\mu\text{m}$, as shown in Figs. S2(E)-(H). The hydrophilic materials contained a high level of peaks and valleys compared to the hydrophobic materials. This characteristic promoted the evaporation/drying rate of the respiratory droplets, changed the stability and morphology of the droplet interface, and created pressure gradients, which affected the evaporative rate and susceptibility of viruses.

The surface layers of enveloped viruses (coronavirus and influenza viruses) contain protein/lipid membranes. Viruses bind to host cells/solid materials through this membrane. Owing to external environmental conditions, virus-containing water droplets on a surface evaporate, leading to dehydration. Thus, viruses can be inactivated via the dehydration of material surfaces. Viruses need water on both sides to remain stable, and dehydration causes changes in the structural protein of the virus. Therefore, viruses persist longer in nonporous materials than in porous materials because nonporous materials do not absorb moisture from materials containing the adsorbed virus.

The coronavirus outbreak posed significant challenges in hospitals, primarily in the usage of PPEs. Viruses and other pathogens in PPEs (different materials made of cotton fabric) must be prevented from infecting patients and healthcare workers. Different sterilization techniques are used; for example, hydrogen peroxide, sodium hypochlorite, and ethanol compounds have been effectively applied to sterilize PPEs and other contaminated materials [42]. However, chemical disinfectants (sodium hypochlorite) indirectly promote environmental hazards and also promote chemical-resistant microbes. Hence, alternative methods for controlling pathogens are required. Currently, coronavirus inactivation is accomplished in different ways, including UV LEDs. For example, Bradley [43] used UV LEDs to inactivate SARS-CoV-2 on surfaces and in liquids. These types of methods use high power density and enhance the adhesion or modify the material properties. To avoid this problem, we used nonthermal plasma-generated RONS [16].

The RONS production depends on the plasma device(s) used. RONS are essential components of intracellular signaling, and high concentrations induce oxidative stress and decline the complex network of intracellular biochemical pathways [44]. If a high concentration of RONS produces a toxic effect on the host cell, and PAW is stored for further use for a longer period (up to one year at $-80\text{ }^\circ\text{C}$) [22], the extended storage time changes the chemical substance concentrations, creating toxic effects in the cell. Another study also demonstrated that high concentrations of sodium hypochlorite and RONS can induce cell cytotoxicity, particularly damaging the cell wall, cell membranes, DNA, enzymes, and other proteins [45]. In contrast, plasma-generated RONS mixed with water are used in various fields of application, such as medicine, agriculture, and the environment.

Guo *et al.* [46] reported the inactivation of pseudovirus spike proteins using PAW. Another study by Qin *et al.* [47] discovered that plasma-generated reactive species inactivated the SARS-CoV-2 spike protein receptor binding domain (RBD), and this was responsible for binding to the human angiotensin-converting enzyme 2 (hACE2). All other studies reported that PAW effectively inactivated the viruses, but they all produced smaller volumes of PAW. Our cylinder DBD produced a large volume of PAW, which effectively controlled the HCoV-229E viruses (as shown in Fig. 4).

In this study, we investigated the inactivation of viruses on cotton fabric for different plasma gas exposures and different concentrations of PAW- NO_x generated from three plasma modes (NO_x , O_3 , and a combination of NO_x and O_3). First, we investigated virus inactivation in cotton fabric for various plasma gas exposures, as shown in Fig. 5(B)-(E) (NO_x , O_3 , and combination of NO_x and O_3 , respectively), and for different NO_x

concentrations in the PAW, as shown in Fig. 6(F)-(H) (PAW- NO_x , PAW- O_3 , and PAW- NO_x and O_3 , respectively), as well as in virus-contaminated cotton fabric immersed in PAW, as shown in Fig. 6(I)-(K) (PAW- NO_x , PAW- O_3 , and PAW- NO_x and O_3 , respectively). Without plasma gas exposure (air only), the virus was not significantly reduced within 2 h. The NO_x gas exposure significantly reduced the virus compared to the O_3 and combination of NO_x and O_3 . In hospitals (which contain blood strain or pathogenic microbes) and in homes, NaOCl is used as a disinfectant. However, high concentrations of NaOCl chemically induce damage to the cloth fibers, promoting toxic environmental effects. Cotton fabric was washed with different kinds of bleaches or detergents, these containing chemicals that directly or indirectly induce a harmful impact on the water or aquatic organisms, and soil, and indirectly promoted the human health problem. Chlorine and peroxide-based by-products such as chlorinated organics acid, which persist in the environment and plasma generated RNS, were eco-friendly to control the virus without causing water, soil, and air pollution.

To overcome this problem, we used different optimized concentrations of PAW- NO_x to inactivate viruses in water and on cotton cloth surfaces containing HCoV-229E. Biological compatibility of PAW depends on the concentration and composition of its reactive species. The results indicated that the PAW- NO_x effectively inhibited the HCoV-229E persistence, the exact value of which depended on the NO_x concentration. There is no harm in using highly toxic NO_x (high concentration) to control the virus in the hospital, and at the same time, there is no need to worry about the toxicity in the materials. However, for in vitro testing, the toxicity and safety level of cotton fabric need to be analyzed. Less or safe levels of PAW reduce the virus titer and also reduce the cotton fabric coming out, irrigation and tissue damage in the hospital environment. This cytotoxicity clearly showed that the PAW treatment is biocompatible for washing. After the viruses were inactivated, immunofluorescence and fluorescence-activated cell sorting (FACS) analyses indicated that the spike protein intensity and the membrane and enveloped mRNA expression levels were reduced compared to those of the control group (as shown in Fig. 7). Therefore, cold plasma is substantially safer than other treatment methods (e.g., NaOCl), as it can ensure safe virus inactivation in hospitals.

4.1. Virus inactivation mechanisms

Viruses on material surfaces persist for hours to days, depending on the environmental factors (e.g., temperature and relative humidity) and the porosity of the materials. Hydrophilic and hydrophobic materials containing the virus absorb liquid into the material, and this desiccation improves the virus' efficiency [12] because water molecules have an incredibly strong polarity [48]. The interaction between the viral surface protein and the surrounding water is often called the protein hydration layer (it is sometimes called the hydration shell) and is the basis of the stable structure [49]. This binding supports viral protein hydration and strong viral protein binding in hydrophilic materials (e.g., paper, cotton fabric, wood, and masks), as water molecules fill the space between the viruses. In hydrophobic materials (e.g., stainless steel, glass, and plastic), virus interaction is significantly weak (with the exception of copper), and the expansion of water drops is too insignificant to yield even inter-gaps between the viruses and material (Fig. 8A). The DBD plasma generated reactive species that slightly altered the chemical composition of the cotton materials, and at the same time surface of the materials loaded various oxygen-containing polar functional groups such as $-\text{OH}$ and $\text{C}=\text{O}$ [50]. These functionalized polar groups can enhance the hydrophilicity and adhesion properties of cotton fabrics, and this condition holds the PAW for a long time and increases the virus inactivation. Strong material binding increases virus inactivation and weak binding decreases virus inactivation. Key RNS in PAW for virus inactivation on cotton include nitric oxide (NO), nitrite (NO_2^-), nitrate (NO_3^-), and peroxynitrite (ONOO^-). These species disrupt viral proteins and RNA through nitration, S-nitrosylation, and oxidative damage, with

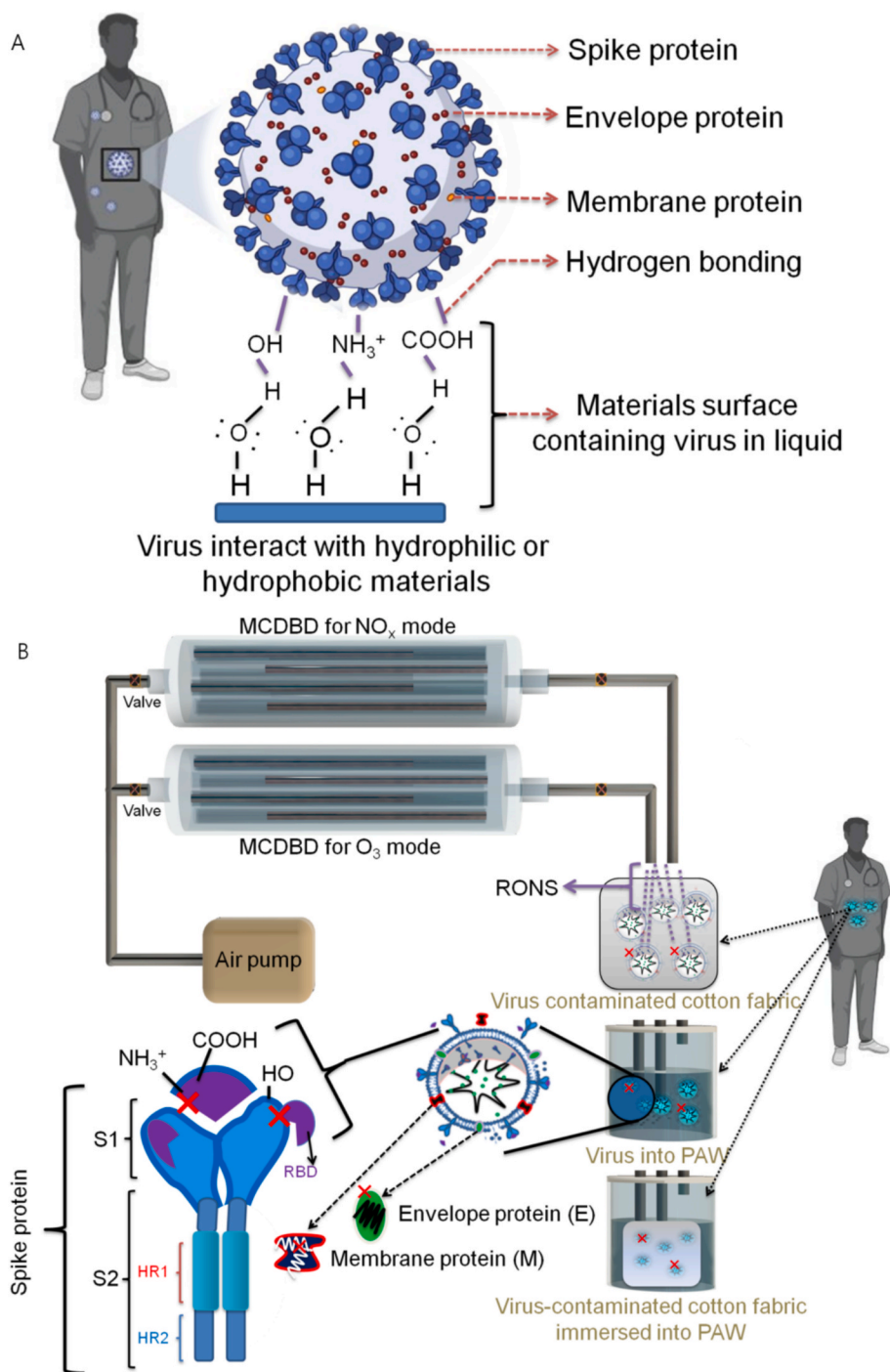


Fig. 8. Feasible mechanism of virus inactivation in solid materials: (A) virus interactions with solid materials (PPEs), (B) MCDBD plasma-generated RNS inactivates the virus in three modes (plasma gas, PAW, and virus-infected cotton fabric immersed in PAW), and also reduces the expression of the S, E, and M genes. For diagram making partly we used Bio render.

ONOO^- being particularly effective. Cotton's porous nature enhances RNS retention, boosting antiviral efficacy. These species inactivate viruses on cotton fabrics through multiple pathways: disruption of lipid envelopes, oxidative modification of viral surface proteins, and damage to viral RNA or DNA. Cotton's porous structure facilitates deeper penetration and localized accumulation of reactive species, enhancing the efficacy of viral inactivation.

Joonaki *et al.* [51] explained the interactions between the viral surface protein and the chemical nature of the materials. The connection between SARS-CoV-2 viral proteins and solid surfaces at various pH

values is below the isoelectric point. The NH_2 , NH_3^+ , COOH , and COO^- groups of amino acids in the spike protein attached to the surface of SARS-CoV-2 were connected to the positive charge that mediated solid adsorption through electrostatic interactions and hydrogen bonding. Therefore, a new approach is required to inactivate coronaviruses on a surface via cold atmospheric plasma (CAP). Different types of plasma devices have been used to control viruses; for example, DBD plasma-generated ozone reduced the HCoV-229E virus titer by 2 and 3.4 $\text{TCID}_{50}/\text{mL}$ under ozone concentrations of 10 ppm and 80 ppm, respectively [52]. Also, virus stability is affected by the interactions

between viral surface proteins (such as S, E, and M) and material surfaces.

It has been mentioned earlier that plasma-generated RNS in gaseous phase, particularly NO_x (NO and NO₃), interact with viral proteins, altering the amino acid sequencing, such as 2-oxo-histidine, or inhibiting the palmitoylation of the spike protein [53,54]. While in aqueous phase RNS, which primarily include nitrite (NO₂⁻) and nitrate (NO₃⁻) ions, promotes the deactivation of virus proteins. In our PAW, these stable RNS are predominant and play a significant role in antiviral activity, along with short-lived species such as peroxynitrite (ONOO⁻), when generated transiently. This type of protein alteration forces structural changes in the viral elements, which lead to virus inactivation. Tizaoui [55] reported that O₃ devastated the cysteine residue content in the glycol and spike proteins in the coronavirus. These cysteine residues were made of sulfhydryl groups called thiol groups (R-SH), and these groups primarily targeted virus entry and viral membrane binding into the host cells. The O₃ oxidizes the thiol groups of sulphonic acid (R-SO₃-H) in the cysteine unit of the spike protein owing to the sulfate modification in the R-S-S-R, which is an inability to bind the host cell surface containing an angiotensin-converting enzyme 2 (ACE2) receptor [56]. As a result of this reaction, the virus titer was reduced in all treatment conditions from 4.57 to 0.64 TCID₅₀/mL.

During PAW generation, the charged particles in the gas phase are mixed and react with water molecules, producing different kinds of RONS. The inhibition mechanism of viruses on cotton fabric treated by MCDBD plasma is shown in Fig. 8(B). The NO_x effectively controlled the HCoV-229E. Dario Akaberi *et al.* [57] studied NO donor S-nitroso-N-acetyl penicillamine (SNAP) to reduce SARS-CoV-2 replication and viral RNA at different concentrations (200 μM and 400 μM). The coronavirus contains four typical amino acids: cysteine, tryptophan, tyrosine, and methionine. Plasma-generated RONS, ONOO⁻, and O₂⁻ promote oxidation to tyrosine, tryptophan, and histidine located at the virus receptor binding domain (RBD) and N-terminal domain (NTD), which may impair the ability of the RBD to bind to the cell receptor angiotensin converting enzyme-2 (ACE2) and the function of NTD. Similarly, PAW-NO_x interacts with the cysteine protein on the coronavirus surface and forms a disulfide bond with S-nitrosothiols (SNOs). Not only that, but cysteine also modifies thiol (-SH) groups, while at the same time altering this disulfide bond during plasma treatment, and due to this reaction viruses are inactivated.

5. Conclusions

Human coronavirus-229E (HCoV-229E) is persistent for up to 19 days depending on the materials involved, and various disinfectants (NaOCl) are used to control the virus on the contaminated surfaces. In hospitals, NaOCl compounds are used as a disinfectant and bleach. However, high concentrations of NaOCl damage the quality of cotton in PPEs and can also induce skin and eye irritation in patients or workers. To prevent this, we used PAW to treat surfaces contaminated with HCoV-229E. Nonthermal atmospheric plasma gas and PAW were extensively evaluated for their performance in inactivating viruses in cotton fabrics. Toxic and non-toxic concentrations of PAW-NO_x were used to validate the antiviral efficacy on cotton fabric. However, high concentrations of PAW damage human fibroblast lung cells. Nevertheless, the results indicated that non-toxic doses of PAW-NO_x required less time to significantly inactivate viruses compared to the untreated (control) group. In all three experimental conditions, the viruses were effectively inactivated. Owing to the plasma treatment, the expression of viral S, E, and M proteins decreased. Hence, it is necessary to further develop effective MCDBD plasma exposure methods to control the spread of coronavirus in hospitals as well as in open places, either by using plasma gas or PAW. In hospital washing detergent or NaOCl price is 1.4 USD per liter but our PAW production cost is less than 1 USD, because we used tap water and consume less than 300 V power to produce PAW. NTP technology for virus inactivation in solid materials

is at an early stage. We need further exploration, particularly which type of RONS interacts with materials and virus inactivation depends on the chemical nature of the materials or RONS. However, the need for future direction is how to improve higher virus efficacy in the solid materials, the particular modes of action, and the plasma functionality in scaled-up systems remain largely unknown. Scaleup the PAW and virus inactivation, by increasing the airflow rate of MCDBD plasma and slightly varying the power and voltage, there is a greater chance to MCDBD plasma generated PAW uses in the healthcare environment. Moreover, we need to identify which type of RONS particularly other than NO_x is involved in virus inactivation. The future research direction would be a combination of NTP with already existing hospitals using conventional washing methods (chlorine-based or other chemical), have a synergetic outcome on virus efficacy, reduce the treatment time, low energy consumption, and less environmental problems.

CRedit authorship contribution statement

Sekar Ashokkumar: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jun Sup Lim:** Validation, Formal analysis. **Jinsung Choi:** Validation, Formal analysis. **Shaik Abdul Munnaf:** Formal analysis. **Ihn Han:** Validation. **Neha Kaushik:** Formal analysis. **Nagendra Kumar Kaushik:** Validation, Methodology, Supervision. **Yung Oh Shin:** Methodology, Investigation. **Eun Ha Choi:** Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cej.2025.163721>.

Data availability

Data will be made available on request.

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