Inactivation effect of plasma-generated reactive oxygen and nitrogen species on human coronavirus

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Abstract: To assess a potential of human coronavirus inactivation using atmospheric pressure plasma (APP) technology, HCoV-229E virus, one of seven species of human coronaviruses, was exposed to various reactive oxygen and nitrogen species (RONS) generated by APPs. As a result, aqueous superoxide anion radicals ($O_2^{\bullet-}$) and/or $O_2^{\bullet-}$-derived species, and gaseous dinitrogen pentoxide (N_2O_5) emerged as promising candidates for efficient virus inactivation using APP.

Keywords: Atmospheric pressure plasma, Human coronavirus, Superoxide anion radicals, Dinitrogen pentoxide

1. Introduction

The recent global pandemic of Corona Virus Disease-19 (COVID-19), caused by infection with a novel coronavirus, SARS-CoV-2, is producing a growing demand for a powerful virus inactivation method. To date, seven species of human coronaviruses which infect humans have been identified: HCoV-NL63, HCoV-229E, HCoV-HKU1, HCoV-OC43, SARS-CoV, MERS-CoV and SARS-CoV-2 [1]. Compared to the SARS-CoV and SARS-CoV-2, four of these viruses, including HCoV-NL63, HCoV-229E, HCoV-HKU1 and HCoV-OC43, are less pathogenic and can cause common cold and self-limiting respiratory infections, which account for 15%–30% of common cold cases [1]. In particular, HCoV-229E was isolated in 1966 [2] and has been well studied so far.

Many studies have ever showed efficient bactericidal and/or virus-inactivating effects of non-equilibrium atmospheric pressure plasma (APP) on various bacteria and viruses [3]. These also indicated significant roles of reactive oxygen species (ROS) such as ozone (O_3), singlet molecular oxygen ($^1O_2$), superoxide anion/ hydroperoxyl radicals ($O_2^{\bullet-}$/$HO_2^{\bullet}$), hydroxyl radical ($^{\bullet}OH$) in the APP-induced inactivation. On the other hand, the study on coronavirus inactivation by non-equilibrium APP is still scarce and the inactivation potential and mechanism remain unclear.

In this study, inactivation effects of plasma-generated RONS on HCoV-229E virus were investigated using a humidified helium (He + H_2O) APP and a selective gaseous RONS [dinitrogen pentoxide (N_2O_5), O_3, nitric oxides (NO/NO_2)] production system based on two APP reactors.

2. Experimental

The He + H_2O APP is generated as shown in Fig. 1(a) [4]. Humidified helium gas serves as the working gas, with its flow rate (***f***) through the quartz tube regulated by a mass flow controller, and typically, ***f*** = 3 L/min. The high-voltage (8.7 kV_{p-p}) with a frequency of 8.07 kHz is applied between the two electrodes; the powered electrode is a 1.5-mm diameter tungsten rod and the other is a grounded electrode plate, generating the He+H_2O APP plume in contact with the solution. After the He+H_2O APP exposure, $^{\bullet}OH$, H_2O_2, NO_2^- were characterized using chemical probes (terephthalic acid, Trinder's reagent and Griess reagent). Details of the experimental conditions can be found in [4].

Figure 1(b) shows an experimental setup for HCoV-229E virus inactivation using the selective gaseous RONS production system based on two APP reactors. This plasma system can selectively generate N_2O_5, O_3, and NO/NO_2 gas. Briefly, N_2O_5 can be generated by mixing the gases from two independent plasma reactors; 1. low gas temperature reactor (LT plasma reactor) for selective O_3 generation and 2. high gas temperature reactor (HT plasma reactor) for selective NO/NO_2 generation. O_3 and NO/NO_2 can be generated simply with electric switching for the HT and LT plasma reactors. Details of the device/method can be found in [5].

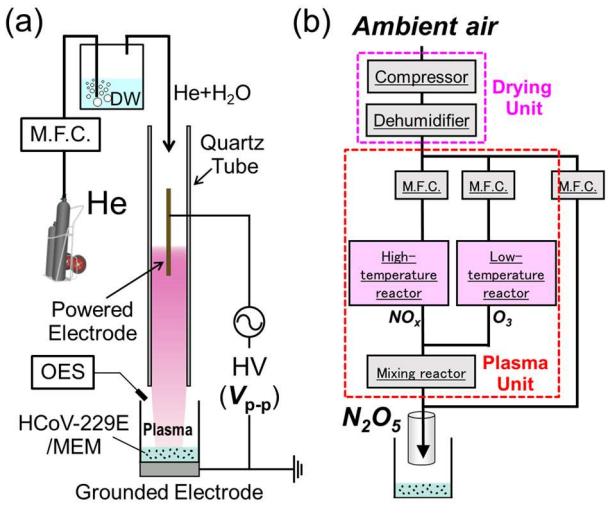

Fig. 1. The experimental setup for (a) He + H_2O APP exposure and (b) gaseous N_2O_5 exposure to HCoV-229E virus solution.

To detect the virus-inactivating effects of the direct APP exposure and the APP-generated RONS, HCoV-229E solution was directly exposed to the He + H_2O APP plume or the gaseous RONS (N_2O_5, O_3, NO_x), and the virus infectivity was measured by end-point dilution assay according to the methods described in a previous report [4, 6].

3. Results and Discussion

Summary of generation rates of reactive species by the He-APP exposure is shown in table 1. Generation rates of $^{\bullet}OH$, H_2O_2 (including H_2O_2 precursors such as $^{\bullet}OH$), NO_2^- (including NO_2^- precursors) were 1.7, 9.2, and 3.3 nmol/s, respectively.

Table 2 shows inactivation effects of the He + H_2O APP exposure and the APP-generated N_2O_5 gas exposure on HCoV-229E virus. The He+H_2O plasma exposure for 30 s significantly reduced the titers of HCoV-229E by 3 $log_{10}TCID_{50}$ /mL, indicating that the plasma treatment successfully inactivated the HCoV-229E virus. N_2O_5 gas exposure also inactivated the HCoV-229E virus in a dose-dependent manner and the inactivation effect for 120-s treatment was roughly comparable to the plasma plume treatment for 30 s. Thus, N_2O_5 can be a promising candidate for efficient human coronavirus inactivation.

Generally, direct plasma plume exposure delivers a complex of UV light, charged particles, and reactive species to the target. Then, candidate factors in the APP-induced virus inactivation are as follows; 1. Long-lived RONS (*e.g.* H_2 O_2 and NO_2^-), 2. short-lived RONS (*e.g.* $^{\bullet}OH$, $^1O_2$, $O_2^{\bullet-}$, $^{\bullet}NO$), 3. mechanical stress (*e.g.* fluid stress), 4. photostress (*e.g.* UV light), and 5. thermal stress. On 1. long-lived RONS, H_2O_2 alone, and an admixture of H_2O_2 and NO_2^- (the H_2O_2 and NO_2^- concentrations were adjusted to equivalent for the APP exposure for 30 sec) did not significantly reduced viral titers of the HCoV-229E. Thus, we concluded that long-lived RONS cannot be responsible for the APP-induced virus inactivation.

Next, contributions of 2. short-lived RONS were examined using several scavengers [D-mannitol and N-acetylcysteine (NAC) for $^{\bullet}OH$, histidine (His) for $^1O_2$, carboxy-PTIO for $^{\bullet}NO$, SOD for $O_2^{\bullet-}$]. As a result, only SOD among those scavengers was significantly effective for the recovery of the APP-induced decrease in the viral titers as shown in Table 3. Thus, $O_2^{\bullet-}$ and/or $O_2^{\bullet-}$-derived species seems to be one of key factors in the APP-induced virus inactivation. However, the recovery of the viral titers by the addition of SOD was not full, which implies potential contributions of other RONS and/or factors other than RONS such as 3. mechanical stress, 4. photostress, and 5. thermal stress.

In conclusion, the He + H_2O APP plume exposure successfully inactivated the HCoV-229E virus and $O_2^{\bullet-}$-related chemical reaction in a network of interconnected reactions induced by the APP exposure can be very important for the APP-induced virus inactivation. On the other hand, N_2O_5 gas exposure successfully inactivated the HCoV-229E virus without plasma plume contact. This effect was clearly due to N_2O_5-induced chemical inactivation and N_2O_5 might be one of key factors for a more efficient virus inactivation method using APP.

Table 1. Generation rates of reactive species by the He APP exposure [4].

Reactive species	$^{\bullet}OH$	H_2O_2	HNO_2 /NO_2^-
Generation rate (nmol/s)	~1.7	9.2	3.3

Table 2. Inactivation effects of the He + H_2O APP exposure and the APP-generated N_2O_5 gas exposure on HCoV-229E virus.

Treatment	He plasma 30 sec	N_2O_5 gas 60 sec	N_2O_5 gas 120 sec
Decrease in viral titer ($Log_{10}TCID_{50}$ /mL)	3.5 ± 1.2	1.3 ± 0.4	2.8 ± 0.4

Table 3. Effects of various RONS scavengers on recovery of viral titers decreased by the He + H_2O APP exposure for 30 sec [4].

Scavenger	D-man	NAC	His	c-PTIO	SOD
Target species	$^{\bullet}OH$	$^{\bullet}OH$	$^1O_2$	$^{\bullet}NO$	O_2^-
recovery of Viral titer ($Log_{10}TCID_{50}$ /mL)	< LOD	< LOD	< LOD	< LOD	1.5 ± 0.4

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