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Effect of chlorine dioxide on avian influenza A (H7N9) virus

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ABSTRACT

Avian influenza remains a threat to human wellbeing. Hypochlorite derivatives are commonly used as disinfectants to prevent the spread of the disease. The World Health Organization (WHO) has listed chlorine dioxide (ClO_2) as an A1-level, safe, and efficient disinfectant. In this study, we tested the efficacy of ClO_2, in aqueous solution and gas forms, against avian influenza A (H7N9) virus. The virus suspension was mixed with ClO_2 aqueous solutions of various concentrations and for various time intervals. Aliquots of the mixture were then serially diluted, and the 50% tissue culture infective dose ($TCID_{50}$) was measured with a hemagglutination test on MDCK cells. ClO_2 gas produced from generators was introduced in a chamber containing the virus suspension in a Petri dish. The infective activity of the surviving virus was measured by the hemagglutination test. An aqueous solution of ClO_2 at 126 μg/mL for 15 s was effective given that no surviving virus was detected with the hemagglutination test. ClO_2 gas at >5 μL/L sustained for 1 h inactivated the virus effectively, while at 2.5 μL/L for 1 h, it only partially inactivated the virus. ClO_2 as gas or aqueous solution at a certain concentration is effective in inactivating the H7N9 virus, and can be applied for the decontamination and disinfection of environments.

1. Introduction

Infections with avian influenza A viruses (H5N1, H5N6, H6N1, H7N4, H7N9, H9N2, and H10N8) in humans have recently been identified. Among them, H7N9 and H5N1 are highly pathogenic to humans, and are distinct from seasonal influenza viruses. As zoonotic agents transmitted by droplets, aerosol, and direct or indirect contact, they cause respiratory diseases with a wide range of manifestations, from asymptomatic or mild to severe disease and death [1]. After an outbreak of H5N1 virus in Hong Kong, China (1997), severe respiratory infections with avian influenza A (H7N9) virus were reported by the World Health Organization (WHO) in humans in Shanghai and Anhui, China, in 2013 (WHO, A/Shanghai/4664 T (H7N9)) [2–4]. Most people infected with H7N9 virus had recent exposure to live poultry or environments potentially contaminated by live birds. Since then, the cases of H7N9 virus infections have increased rapidly in Zhejiang, Jiangsu, and Guangdong Province as the disease spread to the rest of China [5,6]. Other countries, such as Canada and Malaysia, have reported cases of human infection with H7N9 virus, mostly imported from Chinese travellers [7,8]. Birds can carry H7N9 virus in their secretions or excretions. It is speculated that the transmission route is via the respiratory tract, or direct contact with the virus, or by close contact with infected poultry secretions or excretions. High-risk groups mainly include individuals engaged in poultry farming, sales, slaughter, and processing industries, and those who have been in contact with poultry within one week prior to disease onset [2]. Some pandemic influenza viruses are reportedly stable at near neutral pH or cold temperatures, or in water of low salinity [9], although those characteristics may not apply to all strains of influenza viruses.

To prevent the spread of pathogens and to control the disease, disinfectants are used to decontaminate poultry farm markets, items used by patients, or the surrounding environment. The purpose of disinfectants is to block transmission pathways, so as to prevent the spread of infection. Disinfectants are classified as low-efficiency, medium-efficiency, or high-efficiency. High-efficiency disinfectants, such as those containing chlorine, glutaraldehyde, and hydrogen peroxide, can kill all bacterial propagules (including mycobacteria), viruses, fungi, fungal spores, and bacterial spores. Disinfectants containing

Abbreviations: $TCID_{50}$, 50% tissue culture infective dose; μg/mL, microgramme per microlitre; BSA, bovine serum albumin; mo, month; d, day; w/v, weight/volume; v/v, volume/volume.

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HIGHLIGHTS

Scientific question

H7N9 virus is highly pathogenic transmitted by droplets, aerosol, or direct or indirect contact. For controlling H7N9 disease, it is important to select effective disinfectants using to decontaminate poultry farm markets, items used by patients, or their surrounding environment.

Evidence before this study

Chlorine dioxide (ClO_2) is a globally recognized microbicide disinfectant, and it is new-generation, safe, efficient, broad-spectrum, and powerful, with strong oxidizing activity. It disrupts bacterial cell walls and damages viral capsid proteins and viral RNA

New findings

In the study, an aqueous solution of ClO_2 at 126 g/mL for 15 seconds was effective given that no surviving virus was detected with the hemagglutination test. ClO_2 gas at >5 L/mL sustained for 1 h inactivated the virus effectively

Significance of the study

Our findings reveal that ClO_2, as a gas or an aqueous solution at a certain concentration, is effective in inactivating the H7N9 influenza virus, and can be applied for decontamination and disinfection of environments.

chlorine (such as sodium hypochlorite, calcium hypochlorite, chlorinated trisodium phosphate, and chlorine dioxide) are high-efficiency disinfectants dissolved in water. Some of them produce hypochlorous acid with a broad spectrum of microbicidal activity, and are often described in terms of their free available chlorine. Chlorine dioxide (ClO_2) is a globally recognized microbicide disinfectant; it is a new-generation, safe, efficient, broad-spectrum, and powerful disinfectant with strong oxidizing activity [10–12]. ClO_2 gas was registered as a disinfectant by the U.S. Environmental Protection Agency in 1988, and since then, it has become popular in healthcare industry and biomedical research. It is a potent oxidizer, which disrupts bacterial cell walls and damages viral capsid proteins and viral RNA [13,14]. It is usually generated by adding acid to a sodium chlorite ($NaClO_2$) solution, and it inactivates bacteria, fungi, and viruses [15–18]. When it reacts with organic matter in water, no chloroform or carcinogenic or teratogenic compounds are generated. It is listed as an A1-level, safe, and efficient disinfectant by the WHO [10]. Therefore, chlorine dioxide is the most suitable chlorine disinfectant. The gas form of chlorine dioxide at low concentrations (subtoxic levels) is safe to use in crowded areas, without evacuation of people [19–21]. According to the U.S. Occupational Safety and Health Administration, the long-term (8 h) permissible exposure level to ClO_2 in environmental air in a human workplace is 0.1 μL/L [22].

Chlorine dioxide easily diffuses on the surface of microbial membranes, and can effectively oxidize compounds containing thiol, thereby inactivating microorganisms [23]. Its high diffusion rate and penetration ability in water enhance its long-lasting sterilization properties. It has a wide range of applications, and is commonly used to disinfect tap water, hospital sewage, industrial circulating cooling water, swimming pool water, and aquaculture water. In the present study, we investigated the effects of ClO_2 solution and gas on H7N9 virus.

2. Materials and methods

2.1. ClO_2 solution and ClO_2 gas

Different types of chlorine dioxide used as disinfectants (aqueous solution, gels, and stick cartridge) were formulated to keep ClO_2 stable for long periods, and were produced by Taiko Pharmaceutical Co., Ltd. (Osaka, Japan), as listed in Table 1.

2.2. Virus strain and cell culture

The highly pathogenic avian influenza A (H7N9) virus (A/Shanghai/4664 T/2013) was obtained from Shanghai Public Health Clinical Center and kept in the BSL-3 laboratory of Fudan University. The dog kidney transformed cell line (MDCK) was purchased from the Cell Resource Center of Shanghai Academy of Life Sciences, Chinese Academy of Sciences. MDCK cells were cultured in T25 or T75 flasks (Forma 371, Thermo Scientific Co., Waltham, MS, USA) at 37 °C, 5% (v/v) CO_2, with a DMEM medium containing 4 mM glutamine, 110 mg/L sodium pyruvate, and 4.5 g/L glucose (Corning Cellgro, Manassas, VA, USA) supplemented with penicillin (100 IU/mL), streptomycin (100 μg/mL) (Corning Cellgro), and 10% fetal bovine serum (FBS; Corning, Manassas, VA, USA). MDCK cells were seeded into

Table 1
ClO_2 products tested.

Product	Description	Sample: storage time after being produced	Concentration μg/mL (w/v)
ClO_2 solution, long-lasting type			
Cleverin Spray 60 mL	A ready-to-use, long-lasting gas spray. Ingredients: ClO_2 solution, sodium chlorite solution, surfactant, and silicon defoamer. Manufactured under the patent technology of Taiko Pharmaceutical Co., Ltd. (Japanese patent no. 5757975)	A: 46 months (mo.) and 21 days (d)	87
		B: 33 mo. and 29 d	142
		C: 33 mo. and 9 d	124
		D: 1 day	163
Cleverin Spray 300 mL	The same as above, with a 300 mL solution.	E: 27 mo. and 19 d	135
		F: 25 mo. and 13 d	126
		G: 13 mo. and 3 d	125
ClO_2 gas			
Cleverin Gel60 g	A product that generates ClO_2 gas, based on the patent technology of Taiko Pharmaceutical Co., Ltd. (Japanese patent no. 5593423). It contains ClO_2 solution and a superabsorbent polymer. It can keep a ClO_2 concentration stable for a long time after mixing the liquid and powder and thereafter becoming a gel.	I: 27 mo. and 3 d J: 2 mo. and 4 d	
Cleverin Gel 150 g	The same as above, with a different weight.	K: 32 mo. and 20 d L: 1 mo. and 26 d	
Cleverin G Stick (ClO_2 gas-generating stick)	It generates ClO_2 gas after mixing gelling agent with liquid. (Japanese patent nos. 5593423 and 5645002; by Taiko Pharmaceutical Co., Ltd.)	M: 14 mo. and 7 d	
Cleverin Cartridge (ClO_2 gas-generating cartridge)	It generates a higher concentration of ClO_2 gas after mixing sodium chlorite solution and silica gel impregnated with sulfuric acid (Japanese patent no. 6052508). It can be used in cars, when there is nobody inside.	N: 15 mo. and 25 d	

96-well plates (Corning Costar, Manassas, USA) at a density of 0.8–1.0 × 10^4 cells/well and incubated for 24 h until 80% confluence was achieved for the assay of the viral infection.

2.3. Viral infection and titration

We used diluted 0.25% (w/w) trypsin to disperse the MDCK cells into individual cells, which were counted using a cell counter (Cellometer Auto T4, Nexcelom Bioscience, Lawrence, MA, USA). The cells were then diluted and spread into 96-well plates (0.8× 10^4–1.0 × 10^4 cells/well), placed in a 5% (v/v) CO_2 incubator at 37 °C for 24 h until 60% confluence, and then infected with the virus. H7N9 virus was diluted 10-fold with DMEM medium containing 2.5 μg/mL TPCK-trypsin. After washing the cells twice with 100 μL phosphate-buffered saline (PBS) per well, 100 μL of the diluted virus suspension per well was added to a 96-well plate, and cultured at 35 °C in 5% (v/v) CO_2 for 72 h. The temperature of 35 °C was suitable for influenza virus replication [24].

The avian influenza A (H7N9) virus titer was determined as $TCID_{50}$ by a hemagglutination test. In brief, 1% of chicken red blood cell suspension (50 μL/well) was added to a 96-well round-bottomed polystyrene plate (Coring); then, 50 μL/well of the supernatant fluid from virus-infected cells kept for 72 h was added and set for 60 min.

Agglutination of erythrocytes was evaluated by the naked eye as follows: (1) a layer of erythrocytes evenly distributed on the round bottom was marked as "+ + + +"; (2) erythrocytes that were not precipitated, but the edge was irregular and the area was slightly smaller, were marked as "+ + +"; (3) erythrocytes forming a ring, surrounded by small agglutination, were marked as "+ +"; (4) erythrocyte precipitation that formed small clumps, but without a smooth edge, surrounded by small clots, was marked as "+"; (5) erythrocytes forming a red spot with smooth edges on the bottom in the center of the well were marked as "-". Hemagglutination was observed, and the $TCID_{50}$ value of the virus was calculated by Karber's method. Based on erythrocyte agglutination, the proportion of infected cells was recorded as: -, +, + +, + + +, or + + + +. According to Karber's method, $TCID_{50}$ was calculated as follows: $\log_{10} TCID_{50} = L - d \times (s - 0.5)$, where L is the minimum dilution (1), d is the dilution factor (1), and s is the sum of the infected cells ("+ + + +": 1.00; "+ + +": 0.75; "+ +": 0.50; "+": 0.25; "−": 0).

2.4. Effect of ClO_2 aqueous solution on avian influenza A (H7N9) virus

A ClO_2 aqueous solution commercially sold as a disinfectant was obtained from Taiko Pharmaceutical Co., Ltd. (Osaka, Japan). This product (Cleverin) is formulated to keep the concentration stable for long periods. The solution (150 μL of Cleverin Spray, a long-lasting concentration solution) was added to 150 μL of the H7N9 virus suspension (5.32 $\log_{10}$ $TCID_{50}$/100 L), and incubated for 15 s, 30 s, 60 s, 120 s, or 180 s, and then 450 μL of neutralizer (DMEM medium containing 5 mM sodium thiosulfate and 0.2% bovine serum albumin) was added. The mixture was serially diluted 10-fold (as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}) and inoculated onto MDCK cells; each dilution was tested in triplicate wells and cultured at 35 °C in 5% (v/v) CO_2 for 72 h. The surviving virus was quantitated by $TCID_{50}$ with the hemagglutination test. The MDCK cells were treated with DMEM medium plus the neutralizer as a negative control. Three independent experiments were carried out with each sample.

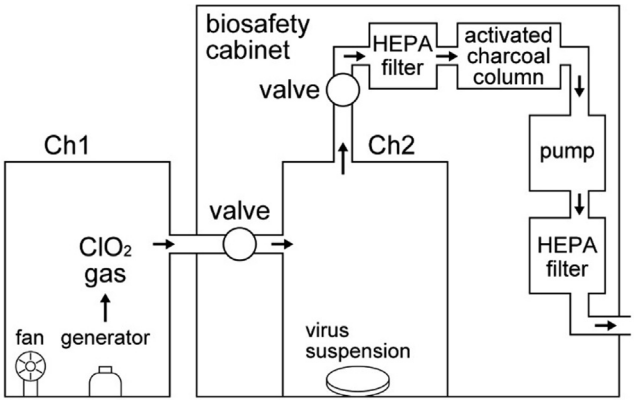

Fig. 1. Diagram of the ClO_2 gas generation and disinfection test. Procedures for testing gas on the H7N9 virus were as follows. In step 1, the test sample was put into a 20 L stainless steel sealed chamber (Ch1). In step 2, ClO_2 gas was generated for 24 h and pumped into another large sealed chamber (Ch2), in which gas concentration was determined. In step 3, 100 μL of influenza A (H7N9) virus suspension in a petri dish was put into Ch2, and exposed to ClO_2 gas for 1 h. In step 4, the surviving virus ($TCID_{50}$) was quantitated by hemagglutination.

2.5. Effect of ClO_2 gas on avian influenza A (H7N9) virus

The chlorine dioxide gas was generated as indicated in Fig. 1. After mixing the liquid and powder in a can, the mixture gradually became a gel, generating ClO_2 gas with a stable concentration for a long time, with Cleverin Gel 60 g, Cleverin Gel 150 g, Cleverin G Stick, or Cleverin Cartridge, as listed in Table 1. In step 1, the tested sample was put into a 20 L stainless steel sealed chamber (Ch1). In step 2, gas was generated for 24 h and pumped into another large sealed chamber (Ch2), in which ClO_2 gas concentration was measured by a detector tube (Gastec No. 23L, Gastec, Ayase, Kanagawa, Japan) by drawing 100 mL of gas-containing air from the chamber for 2 min. In step 3, 200 μL of the H7N9 virus suspension in a Petri dish (6 cm diameter) was put into the Ch2 and exposed to the gas for 1 h. In step 4, the virus was neutralized to ClO_2 with 450 μL of DMEM medium containing 5 mM sodium thiosulfate and 0.2% (w/w) BSA. The surviving virus was quantitated by $TCID_{50}$ with the hemagglutination test. Three independent experiments were carried out with each sample.

3. Results

To determine the effect of the ClO_2 solution on avian influenza A (H7N9) virus, seven samples were tested (samples A to G, listed in Table 1). For each solution, 150 μL was added to a 150 μL virus suspension (A/Shanghai/4664 T/2013, 5.32 $\log_{10}$ $TCID_{50}$/10 μL), incubated for 15, 30, 60, 120, or 180 s, and then neutralized with 5 mM sodium thiosulfate plus 0.2% BSA. The mixture was serially diluted 10-fold and inoculated onto MDCK cells; viral $TCID_{50}$ was determined by hemagglutination (three wells per dilution, repeated three times). After treatment for 15 s with the samples B (142 μg/mL), D (163 μg/mL), E (135 μg/mL), F (126 μg/mL), or G (125 μg/mL, listed in Table 1), no viable virus was detected. Treatment with the sample C (124 μg/mL) for 15 s led to a decrease in the virus titer to 2.80 $\log_{10}$ $TCID_{50}$; after extending the treatment time to 120 s, no virus was detected. Treatment with the sample A (87 μg/mL) for 15 s reduced the viral titer from 5.32 to 4.47 ($\log_{10}$ $TCID_{50}$); after extending the treatment time to 180 s, 2.89 $\log_{10}$ $TCID_{50}$ virus survived. As a negative control, H7N9 virus was treated with DMEM for 180 s, and the titer remained stable at 5.19 $\log_{10}$ $TCID_{50}$. For ClO_2 solution, 1 μg/mL amounts to 1 ppm.

Then, we tested the effect of ClO_2 gas (Gel 60 g, Gel 150 g, G stick, and Cartridge with samples I to N) (Table 1) on avian influenza A (H7N9) virus. The gas was produced by a generator in a sealed chamber (20 L) for 24 h, as shown in Fig. 1. The gas concentration was determined by the detector tube in 100 mL of gas-containing air from the chamber for 2 min. Then, 100 μL of H7N9 virus suspension on a dish was put into the chamber for 1 h. After neutralization with 5 mM sodium thiosulfate plus 0.2% (w/w) BSA, the serially diluted mixtures were inoculated onto MDCK cells (three wells per dilution). The surviving virus after treatment was quantitated by $TCID_{50}$ with hemagglutination. After generation by sample Gel 60 g (I, J), Gel 150 g (K, L), and Cartridge (N) for 24 h, ClO_2 gas concentration was over 5.0 μL/L, whereas sample G stick (M) only reached 2.5 μL/L (Table 2). After exposure to ClO_2 gas (>5.0 μL/L) for 1 h generated by Gel 60 g and Gel 150 g samples (I, J, K, L), the viral titer dropped to an undetectable level. However, exposure to ClO_2 gas (2.5 μL/L) generated by G stick (M) led to a reduction in the viral titer ($\log_{10}$ $TCID_{50}$) from 5.70 to 4.33. ClO_2 gas generated by the Cartridge sample

Table 2
Effect of ClO_2 aqueous solution on avian influenza A H7N9 virus.

Samples[**]	ClO_2 concentration (μg/mL)	Virus titers (log_{10} $TCID_{50}$) after treatment with ClO_2 aqueous solution for time(s)[*]				
		15	30	60	120	180
A	87	4.47 ± 0.58	4.13 ± 0.29	4.05 ± 0.25	2.97 ± 0.14	2.89 ± 0.29
C	124	2.80 ± 1.13	2.63 ± 1.12	2.47 ± 1.18	UD[***]	UD
G	125	UD	UD	UD	UD	UD
F	126	UD	UD	UD	UD	UD
E	135	UD	UD	UD	UD	UD
B	142	UD	UD	UD	UD	UD
D	163	UD	UD	UD	UD	UD
DMEM Control	0	/	/	/	/	5.19 ± 0.24

[*] The solution was added to H7N9 virus (5.32 log_{10} $TCID_{50}$/100 L), and incubated for 15 s, 30 s, 60 s, 120 s, or 180 s, and then 450 L of neutralizer was added. It required 15 s at least for finishing the operation. The mixture was serially diluted 10-fold, inoculated onto MDCK cells, and cultured for 72 h. The surviving virus titer was quantitated by $TCID_{50}$ with the hemagglutination test, and each dilution was tested in triplicate wells. The virus was treated with DMEM medium plus the neutralizer as a negative control. Log_{10} $TCID_{50}$ is shown as the mean ± standard deviation. It needed 15 s at least for finishing the operation procedure.
[**] Prepared ClO_2 solution samples and times: A (46 months and 21 days), B (33 months and 29 days), C (33 months and 9 days), D (1 day), E (27 months and 19 days), F (25 months and 13 days), and G (13 months and 3 days). Three independent experiments were carried out with each sample.
[***] UD: under a detectable level.

Table 3
Effect of ClO_2 gas on avian influenza A (H7N9) virus.

Generators of ClO_2 gas[**]						
Product [*]	Gel 60 g		Gel 150 g		G Stick	Cartridge
Sample ID	I	J	K	L	M	N
Storage period of the sample (mo) [***]	27	2	32	1	14	15
Gas concentration(μL/L [v/v])	>5	>5	>5	>5	2.5	>5
Virus titer (log_{10} $TCID_{50}$) after treatment[***]	UD	UD	UD	UD	4.33 ± 0.19	UD
Virus titer control(log_{10} $TCID_{50}$)	5.70 ± 0.05					

[*] ClO_2 gas generators: The Cleverin products (Gel 60 g, 150 g, G Stick, and Cartridge) consist of salt powder and solution. The two ingredients are kept for a period of time (months). After mixing liquid and powder in a can, the mixture gradually becomes a gel, generating ClO_2 gas to a stable concentration.
[**] Gel 60 g and Gel 150 g are ClO_2 slow-release sterilization deodorants; G Stick is a ClO_2 gas-generating stick, and Cartridge is a higher-concentration ClO_2 gas-generating cartridge. Three independent experiments were carried out with each sample.
[***] The virus titer was quantitated by $TCID_{50}$ with the hemagglutination test, and each dilution was tested in triplicate wells. Log_{10} $TCID_{50}$ is shown as the mean ± standard deviation. UD: under a detectable level.

exceeded 5.0 μL/L (v/v) in 5 min, and no virus was detected after the exposure to the gas for 1 h (see Table 3). For ClO_2 gas, 1 μL/L amounts to 1 ppm.

4. Discussion

The avian influenza virus belongs to the genus *Influenza* A of the Orthomyxoviridae family. These viruses mainly infect birds and poultry, such as chickens and ducks. The viral genome is segmented in eight fragments as single-stranded and negative strand RNA [2]. Based on the different antigenicity of the envelope proteins, i.e., hemagglutinin (H) and neuraminidase (N), the viruses are divided into 16 H subtypes (H1–H16) and nine N subtypes (N1–N9). The subtypes of avian influenza viruses that can infect humans are H7N9, H5N1, H9N2, H7N7, H7N2, and H7N3 [3]. In the present study, both the gas form and the aqueous solution of ClO_2 were demonstrated to be effective in inactivating H7N9 virus. Since ClO_2 gas can dissolve in water, its disinfecting efficacy may result from acting as an aqueous solution. However, it is worth noting that Morino demonstrated the effect of gas against microbes adhering to a hard board [25], which suggests that ClO_2 gas may be effective against influenza virus attached to the surfaces of materials.

ClO_2 inactivates proteins by the oxidation of their tryptophan and tyrosine residues [12]. For instance, protection against influenza A virus H1N1 infection in mice was provided by ClO_2 gas at 0.03 μL/L (v/v) [26]. Ogata and Shibata demonstrated that the mechanism behind protection against infection in mice involved the inactivation of the influenza virus. They further demonstrated that the mechanism was oxidative modification of tryptophan 153 residue (Trp153, W153 in short) in hemagglutinin protein, which the virus needs in order to bind to a cell receptor [27]. Trp153 is present in a receptor-binding domain of hemagglutinin of the virus, and is conserved among many influenza viruses [28]. Trp153 corresponds to Trp160 of hemagglutinin protein in the H7N9 virus (UniProt registration number M9QPD1 for A/Shanghai/4664 T/2013(H7N9)). Furthermore, tyrosine residues in proteins are also oxidized by ClO_2 [12]. Tyrosine 106 (Tyr106, Y106 in short) residue is also present in the receptor-binding domain of the hemagglutinin of H7N9 virus (its numbering is also Y106 in H7N9 virus). This residue is conserved in many influenza viruses, and is considered to be important in receptor-binding [28]. Therefore, it is likely that H7N9 virus is inactivated by the oxidation of Trp160 and/or Tyr106, and its infectivity of MDCK cells is abrogated. We speculate that ClO_2, either as an aqueous solution or a gas, inactivates H7N9 virus by oxidizing its hemagglutinin protein.

The solution samples (A–G) in our research were able to maintain the concentration of chlorine dioxide for a long time. The concentration of chlorine dioxide decreased with the prolongation storage time. For example, sample F still maintained 126 μg/mL chlorine dioxide after 25 months of storage. In the present study, the concentration of chlorine dioxide in the solution above 125 μg/mL was able to effectively inactivate the H7N9 virus (~5 log_{10} $TCID_{50}$) by treatment over 15 s. Therefore, for decontamination of H7N9 virus, chlorine dioxide solution can be sprayed onto surfaces of objects and environments, such as in laboratories, hospitals, and live poultry markets, including farming, sales, slaughter, and processing locations.

Gel 60 g and Gel 150 g were ClO_2 gas slow-release disinfectants, which were able to slowly release the chlorine dioxide gas and maintain the concentration in a confined space. When Gel 60 g was stored for 27 months and Gel 150 g was stored for 32 months, generation of ClO_2 gas was still over 5.0 μL/L. G Stick was a higher concentration ClO_2 gas generator. When the ingredients were mixed after being stored for 14 months, chlorine dioxide gas was generated quickly

and reached high concentrations in a short time. However, this concentration could not be maintained for a long time, which explains why after 24 h, the concentration of chlorine dioxide generated by G stick was only 2.5 μL/L and was less effective for inactivating the H7N9 virus. In the present study, we found that ClO_2 gas (>5.0 μL/L) effectively inactivated the H7N9 virus (~5 $\log_{10}$ $TCID_{50}$). However, the concentration of chlorine dioxide could not be continuously tested in the present study; thus, we do not know the minimum concentration of ClO_2 needed for the inactivation of the H7N9 virus. In addition, it is important to investigate the effect of chlorine dioxide on other viruses, such as SARS-CoV-2.

In view of the safety and effectiveness of chlorine dioxide gas, we recommend the chlorine dioxide gas products for air disinfection in confined spaces such as H7N9 virus laboratories and clinical wards. For semi-enclosed environments such as the home, low-release disinfectants are suggested to ensure long-term safety. Higher-concentration ClO_2 gas-generators, such as G stick and Cartridge, are more suitable for 1 h air disinfection in small confined spaces such as cadaver transport bags, biosafety cabinets, and ambulances. For open spaces, such as live poultry markets, ClO_2 gas generators are unsuitable because the effective ClO_2 concentration is difficult to reach.

Taken together, we conclude that ClO_2 can be applied for H7N9 virus decontamination and disinfection of the environment.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

Author contributions

Zhiping Sun: Conceptualization, Formal Analysis, Investigation, Methodology, Resources, Validation, Visualization. **Yun Qian:** Investigation, Methodology, Resources, Validation, Visualization. **Norio Ogata:** Funding Acquisition, Resources, Supervision. **Xia Cai:** Investigation, Methodology, Resources. **Wendong Han:** Investigation, Methodology, Resources. **Youhua Xie:** Conceptualization, Project Administration, Supervision. **Hirofumi Morino:** Resources, Writing – Original Draft. **Koushirou Sogawa:** Resources, Writing – Original Draft. **Takashi Shibata:** Resources, Writing – Original Draft. **Di Qu:** Conceptualization, Formal Analysis, Funding Acquisition, Methodology, Project Administration, Supervision.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bsheal.2021.12.002.

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