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Verification of MA-T Safety and Efficacy Against Pathogens Including SARS-CoV-2

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Matching transformation system (MA-T) is an on-demand aqueous chlorine dioxide solution. It is a disinfectant developed to maximize the safety of chlorine dioxide radical in water and its effectiveness against various microorganisms. In this study, we examined the safety and effectiveness of MA-T for its use in various infectious disease countermeasures, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and consider if MA-T can be implemented in society. To validate the safety of MA-T, we conducted safety tests and efficacy tests in accordance with GLP-based reliability criteria. To evaluate the efficacy, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) confirmation tests against various bacteria, and virus inactivation test against various viruses including SARS-CoV-2 by $TCID_{50}$ method were performed. The results of safety tests showed that MA-T was at least as safe as Japanese tap water. As a result of efficacy tests for microorganisms, MA-T was effective against many bacteria. Efficacy tests for virus showed that MA-T inactivates SARS-CoV-1, Middle East respiratory syndrome coronavirus (MERS-CoV), rotavirus A (RV-A), hepatitis C virus (HCV), dengue virus (DENV), and hepatitis B virus (HBV). MA-T also inactivated 99.98% of SARS-CoV-2, which is equivalent to ethanol for disinfection. MA-T has proven to be a safe and effective disinfectant. MA-T is a next-generation disinfectant that has the potential to be safer and more effective than conventional chlorine disinfectants and other disinfectants. It also proved to be an effective disinfectant against SARS-CoV-2, which is currently causing pandemic all over the world.

Key words MA-T, SARS-CoV-2, disinfectant, chlorine dioxide, microorganism

INTRODUCTION

In 2020, the world experienced a continuing pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Human history has been and will continue to be affected socially, economically, and culturally by infectious diseases. The fight against infection is expected to continue. Infectious diseases are caused by the transmission of pathogens, and disinfection or removal of pathogens has a very important role as a countermeasure.

Chlorine dioxide gas has long been known for its bactericidal and antiviral effects.[1,2] There are many theories about its mechanism of action, including cell membrane destruction,[3,4] protein inactivation,[5] and viral DNA damage.[6] Due to its effectiveness against viruses and bacteria, it has been used not only in water and wastewater treatment, but has also been considered for environmental and food disinfection or medical applications.[7-11] However, chlorine dioxide gas is highly toxic, and the radicals that are responsible for the reaction are highly reactive. The U.S. Occupational Safety and Health Administration (OSHA) has set its exposure limit at 0.1 ppm for 8 h per day (time-weighted average: TWA).[12] Another agent with a radical mechanism of action is hypochlorous acid, which is widely known. While this agent is utilized in many fields such as oxidizers, bleaching agents, topical disinfectants, and disinfectants, it requires careful handling due to its toxicity.

On-demand aqueous chlorine dioxide solution: MA-T is a chemical agent that makes it possible to control the generation of aqueous radicals by the technology of organic catalysts. The active aqueous chlorine dioxide radical collides with surrounding large amounts of water molecules or chlorite ions, and the radicals return to chlorite ions. However, because of the chemical equilibrium, a new active aqueous radical is formed.[13] In this way, when a reactant is present, MA-T supplies the consumed radical while maintaining an equilibrium state and never produce chlorine dioxide gas.

$$4ClO_2^- + 2H^+ \text{ -> -> -> } ClO_3^- + 2\ ClO_2 + H_2O + Cl^-$$

Also, we proved that MA-T attacked the components of the

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respiratory chain only in live bacteria.[14] As a result, MA-T is expected to be a disinfectant that is far safer and has a stronger anti-pathogenic action than conventional chlorine-based disinfectants.

In this study, based on the hypothesis that MA-T is a safe and effective disinfectant, the safety and efficacy of MA-T was validated. The safety of the product was verified through safety and toxicity studies in animals and humans. For efficacy, we evaluated antimicrobial activity and antiviral activity.

MATERIALS AND METHODS

We conducted safety and efficacy studies against various bacteria and viruses using different concentrations of MA-T. MA-T concentrations were confirmed by absorbance, ion chromatography, and titration and shown as concentrations of dissolved $NaClO_2$.

Safety Test To validate the safety of MA-T, we conducted safety tests in accordance with GLP-based reliability criteria. The tests were conducted in laboratory animals, including single oral dose toxicity tests, ocular irritation tests, primary skin irritation tests, continuous skin irritation tests, skin sensitization tests, and acute inhalation toxicity tests. In addition, a chromosome aberration test using mammalian cultured cells, a reverse mutation test using bacteria, patch test in human, and metal corrosion test were conducted. In the single oral dose toxicity study, the LD50 values were calculated. In the primary skin irritation tests, the primary irritation index (P.I.I.) was calculated. Animal tests were conducted with the approval of the animal experiment ethics committee in Drug Safety Testing Center (approval number; IACUCN17268-1, IACUCN14263, IACUCN16210).

Antimicrobial Test Against Bacteria and Fungi Bacteria that require a dedicated culture medium (*Tannerella forsythia*,[15] *Porphyromonas gingivalis*,[16] *Treponema denticola*[17]) were cultured anaerobically according to published methods.[15-17] *Corynebacterium mastitidis* was aerobically cultured in Mueller Hinton II Broth medium (Becton, Dickinson and Company, NJ, USA) supplemented with 5% horse hemolyzed blood at 35°C for 48 h. *Aggregatibacter actinomycetemcomitans* was cultured in BHI medium (Becton, Dickinson and Company, NJ, USA) containing 0.5% yeast extract in an atmosphere containing 5% CO_2. *Propionibacterium acnes* was cultured in BHI medium anaerobically. The other bacterial species were cultured with BHI aerobically. The four species of fungi were cultured with YPD (2% peptone, 1% yeast extract, and 1% D-glucose) at 30°C. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined according to the following procedure. All the procedures were conducted for three times independently.

(1) The bacterial solution (50 µL) grown to appropriate cell density in a dedicated liquid medium was diluted 190-fold with the same liquid medium, mixed, and used as "bacterial solution A."
(2) Dedicated medium, bacterial solution A, and MA-T were added into the 96 wells of microplate.
(3) After proper time of incubation at 37°C (or 30°C for fungi), MIC was confirmed with microplate reader. The bacteria-free liquid medium instead of solution A was used as a control.
(4) Aliquot (10 µL) was collected from the well near the well on which the microorganism is growing, spread on agar plates, and incubated at 37°C (or 30°C for fungi) to obtain MBC.

Test Against Viruses Anti-viral efficacy against SARS-CoV-2 SARS-CoV-1, Middle East respiratory syndrome coronavirus (MERS-CoV) and SARS-CoV-2 (Hu/DP/Kng/19-020), from Kanagawa Prefectural Institute of Public Health were used for this assay. VeroE6 cells (for SARS-CoV-1and MERS-CoV) and VeroE6-TMPRSS2 cells (for SARS-CoV-2) were used as infected cells. Inactivation tests were performed as follows for three times independently.

(1) 30 µL of virus ($TCID_{50}$ = 1 x 10^5) + 30 µL of disinfectant solution or PBS (control)was mixed and incubated for 1 min and 50 µL of the serial 10-fold dilution was added to VeroE6-TMPRSS2 cells (1x10^4 cells/50 µL/well, 96 wells).
(2) After 3 d, the cells were stained with crystal violet and the $TCID_{50}$ was calculated by the Reed-Muench method.[18]

Anti-Viral Efficacy Against Influenza A Virus (IAV) The influenza virus was Type A [Flu, PR8 strain (H1N1)], and the infected cells are MDCK cells (Madin-Darby canine kidney cells). Inactivation tests were conducted for three times independently at the Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University.

(1) Test solution or solvent was diluted to an desired concentration in phosphate buffer [PBS(-) free of Mg^{2+} and Ca^{2+}], and added 50 µL of Flu A stock solution (2 x 10^8 $TCID_{50}$/mL) to 450 µL of each, and incubated at room temperature for 1 min at 25°C.
(2) After the incubation was completed, 50 µL of each mixture was added to 450 µL of PBS (-) with 1% bovine serum albumin, and prepared 10^2- to 10^5-fold dilutions (4°C).

MDCK cells monolayer-cultured in 24-well plates in the Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum and antibiotics at 37°C in the presence of 5% CO_2. After washing once with serum-free DMEM, the MDCK cells were added to DMEM (1 mL) supplemented with 0.1% BSA and 25 µg/mL trypsin. Next, 100 µL of the virus dilution was added and the cells were incubated at 37°C for 4 d.

(3) After incubation, the cells were stained with 2.5% crystal violet solution, washed three times with PBS (-) decolorized, and dried under UV light sterilization.
(4) $TCID_{50}$/mL was determined by the Reed-Muench method.

Anti-Viral Efficacy Against Feline Calicivirus (FCV)

(1) Preparing viral solution
   i. FCV was inoculated into the feline kidney-derived cell line (CRFK cell).
   ii. After adsorption at 37°C for 1 h, the inoculated virus solution was removed and washed twice with sterile PBS.
   iii. Serum-free Eagle's MEM medium was added and cultured at 37°C under 5% CO_2.
   iv. When the cytopathic effect (CPE) of about 70 to 80% was observed (3 d after inoculation), the culture supernatant was collected.
   v. The collected culture supernatant was centrifuged at 3000 rpm for 30 min, and the centrifuged supernatant was dispensed and stored at -70°C or lower as the virus solution.
(2) Confirmation of cytotoxicity

i. Each MA-T solution was serially diluted 10-fold with PBS containing antibiotics.
ii. CRFK cells were inoculated with each test sample diluted 10-fold serially.
iii. On the 5th day after the inoculation, toxicity to CRFK cells was confirmed based on the presence or absence of CPE.

(3) Efficacy test against FCV
i. 10 mL MA-T solution was placed in a test tube and inoculated with one-tenth of a volume of the viral solution.
ii. After incubated for 60 min at 25°C, it was collected and used as the test solution.
iii. The collected test solution was serially diluted 10-fold with PBS containing antibiotics.
iv. CRFK cells were inoculated with a 10-fold serially diluted test solution.
v. On the 5th day after the inoculation, the virus titer was measured based on the presence or absence of CPE.

Efficacy Against Other Viruses Efficacy tests against viruses (Rotavirus A (RV-A), Hepatitis C virus (HCV), Dengue virus (DENV), Hepatitis B virus (HBV)) were conducted for three times independently at the Research Institute for Microbial Diseases, Osaka University. Virus culture supernatant with serum or purified pathogen sample dissolved in PBS was reacted with the MA-T test sample for 60 s, diluted in DMEM medium, and inoculated into susceptible cells to determine residual viral titers.

Day1 Each cell was prepared at 1 x 10^5/mL and seeded into 96-well plates at 100 µL each.

Day2
(1) Cell medium was replaced with 2% FBS DMEM (50 µL).
(2) Virus was adjusted to 1x10^5/30 µL
(3) 30 µL of test sample (if 100 ppm, adjust at 200 ppm) was collected.
(4) Mix (2) and (3) and let them react for 60 s.
(5) Add 240 µL of serum-free DMEM (300 µL in total, diluted 5-fold). Using a mixture, dilution columns were prepared and cells inoculated on day 5. Viral titers were determined at 4 d post-infection.

RESULTS

The results of the various safety tests are shown in Table 1. In a single oral dose toxicity study in rats, no toxicity was detected at 1000 ppm MA-T and the LD50 was greater than 1000 mg/kg. As with results of other safety tests in animals, no toxicity was observed at 100 ppm MA-T. In addition, a safety of 500 ppm was confirmed in the acute inhalation toxicity test and 1000 ppm in the primary skin irritation test. No toxicity was observed at 100 ppm MA-T in the *in vitro* chromosome aberration test, bacterial reverse mutation test, and human patch test. In a metal corrosion test, the level of corrosion was found to be equivalent to that of tap water at 500 ppm MA-T.

The results of the tests against bacteria are shown in Table 2. The experiments were repeated three times for each bacterium or fungus independently, and the MIC and MBC values were the same for all three tests. MA-T showed MICs and MBCs below 50 ppm against many bacteria and fungi. MIC for *Bacillus subtilis* was 12.5 ppm, while MBC could not be detected. MBCs of *Propionibacterium acnes*, *E. coli* O157:H7 and *Fusarium oxysporum* were not measured for the following reasons. *Propionibacterium acnes* showed extremely low value of MIC and we judged that there was no reason to measure MBC at this time and omitted it. *E. coli* O157:H7 is highly pathogenic and can be inferred from non-pathogenic *E. coli*, we decided to measure only MIC. *Fusarium oxysporum* is a plant pathogen and the MIC was sufficiently lower than our expectation, so we omitted the MBC measurement.

The results of the antiviral test against SARS-CoV-2 are shown in Table 3. The test was conducted for three times independently, with the $TCID_{50}$ shown from the representative value. SARS-CoV-2 showed an inhibitory effect of 99.98% by treating with MA-T at a concentration of 50 ppm or more for 1 min.

Table 4 shows the results of antiviral tests against IAV and other viruses. IAV, SARS-CoV-1, MERS-CoV), HCV and DENV showed an inhibitory effect of 98% or more when treated with 100 ppm MA-T for 1 min. HBV showed a 74.5% inhibitory effect by the same treatment. The inhibitory effects on RV-A were 33.3% at 100 ppm for 1 min and 88.9% at 200 ppm for 1 min. All the tests were performed for three times independently, with data shown from a representative test.

DISCUSSION

The pandemic of SARS-CoV-2 caused various problems all over the world, such as shortage of hand disinfectants[19] and misuse of disinfectants.[20,21] Although ethanol is fast-acting[22] and can be used as a hand sanitizer,[23] it may not be sufficiently disinfecting due to volatilization, and flammability needs to be noted. Chlorinated disinfectants have toxicity, including metal corrosion, mucous membrane irritation, and skin irritation,[24,25] and should be used with great caution in their application and use. Povidone-iodine requires prolonged contact with bacteria compared to ethanol[26] and has the potential for chemical burns from prolonged use.[27] Due to their chemical properties, conventional disinfectants can cause serious damage if used incorrectly. Under such circumstances, it is urgent to develop safe and effective disinfectants, and we have focused our attention on MA-T, which enables us to control the generation of aqueous radicals by the technology of organic catalysts.

Results of various safety tests against oral administration, cutaneous, ocular, inhalation, chromosomal abnormalities, mutations, and metal corrosion proved that MA-T can be safely used at least 100 ppm. In addition, based on the LD50 of 1000 mg/kg, the results of the single oral dose toxicity test at 1000 ppm, the primary skin irritation test, and the acute inhalation toxicity test at 500 ppm, it was suggested that MA-T can be safe to use at higher concentrations such as 500 ppm and 1000 ppm. Also, it can be inferred that MA-T is a safe agent regardless of the route of exposure.

The effectiveness of MA-T against various bacteria could be shown from MIC and MBC in various bacteria in our study. The MIC and MBC of MA-T against *Porphyromonas gingivalis*, *Treponema denticola*, *Tannerella forsythia*, *Aggregatibacter actinomycetemcomitans*, and *Streptococcus mutans*, which cause oral diseases, were below 50 ppm. The MIC was 12.5 ppm for *Bacillus subtilis*, a spore-forming bacterium, but MBC could not be determined. This is due to the strong resistance of bacterial spores to disinfectant, and it is speculated that a high concentration of MA-T is required to

Table 1. Safety and Toxicity of MA-T in Animals and Humans

Test	test laboratory	MA-T concentration (ppm)	result	Method and references
single oral dose toxicity: Rat	Drug Safety Testing Center Co., Ltd.	1000	No toxicity	Revision of Guidelines for Toxicity Studies
ocular irritation: rabbit	Japan Food Research Laboratories	100	Not detected	Guidebook for the Manufacture and Sale of Cosmetics and Quasi-Drug Products 2011-12, Guidance for the Safety Evaluation of Cosmetics (2015)
primary skin irritation: rabbit	Drug Safety Testing Center Co., Ltd.	10000	P.I.I: 4.1Medium stimulus	Guidebook for Manufacture and Distribution of Cosmetics and Quasi-drugs 2011-12 and the Guidance for Cosmetic Safety
primary skin irritation: rabbit	Drug Safety Testing Center Co., Ltd.	1000	P.I.I: 0No stimulus	Guidebook for Manufacture and Distribution of Cosmetics and Quasi-drugs 2011-12 and the Guidance for Cosmetic Safety
Continuous skin irritation: guinea pig	Life Science Laboratories, Ltd.	100	No stimulus	"Guidebook for the Manufacture and Sale of Cosmetics and Quasi-Drug Products 2008" and "Guidebook for good laboratory practice of medicine 2010"
Skin sensitization: guinea pig	Life Science Laboratories, Ltd.	100	No skin sensitization	Guidebook for the Manufacture and Sale of Cosmetics and Quasi-Drug Products 2008 and "Guidebook for good laboratory practice of medicine 2010" Maximization test
Acute Inhalation Toxicity: mouse	Drug Safety Testing Center Co., Ltd.	500	No toxicity	Yamashita method※1
Acute Inhalation Toxicity: mouse	Drug Safety Testing Center Co., Ltd.	100	No toxicity	Yamashita method※1
Acute Inhalation Toxicity: mouse	Drug Safety Testing Center Co., Ltd.	50	No toxicity	Yamashita method※1
In Vitro Chromosomal Aberration Test	Bio Research Center Co.	100	No abnormalities	Notification No. 1604 of Evaluation and Licensing Division, Pharmaceutical and Medical Safety Bureau, Ministry of Health, Labour and Welfare. "Guidelines for genotoxicity testing of pharmaceuticals" November 1, 2009.
Bacterial reverse mutation test	Life Science Laboratories, Ltd.	100	Negative	Guidebook for the Manufacture and Sale of Cosmetics and Quasi-Drug Products 2008,Guidebook for good laboratory practice of medicine 2010
Human patch test	Life Science Laboratories, Ltd.	100	No stimulus	Japan Cosmetic Industry Association. Guidance for the Safety Evaluation of Cosmetics 2015. Tokyo, Japan: Yakuji Nippo, Limited. 48-49.Notification number 0413-1 of Pharmaceutical Safety and Environmental Health Bureau, Ministry of Health, Labour and Welfare. Clinical evaluation guidelines for quasi drugs. April 13, 2017.Other references※2,3
Corrosion test of metallic materials	Acenet Inc.	500	The same as tap water	According to company regulations

※1 Yamashita M, and Tanaka J. Pulmonary Collapse and Pneumonia Due to Inhalation of a Waterproofing Aerosol in Female CD-1 Mice. J Toxicol Clin Toxicol. 1995;33(6):631-7.
※2 Takayama K, Yokozeki H, Matsunaga K *et al*. The Japanese guidelines on contact dermatitis. Jpn J Dermatol. 2009;119(9):1757-93.
※3 Sugai T. Cosmetic Safety. Journal of Japanese Cosmetic Science Society. 1995;19:49-56.

expect an effect on spores as with conventional chlorine-based disinfectants.[28-30] However, for *Bacillus cereus*, which also forms spores, MIC and MBC were as low as 2.5 ppm. It is presumed that *Bacillus cereus* used in this test were the vegetative cells, pre-spore-forming bacteria. For experimental reasons, MBC was not measured for *Propionibacterium acnes*, *E. coli* O157:H7, and *Fusarium oxysporum*. However, the bactericidal effect on *E. coli* O157:H7 can be extrapolated from the bactericidal effect on *Escherichia coli*, and the effect on *Propionibacterium acnes* and *Fusarium oxysporum* can be suggested by the low concentration of their MICs. MA-T was effective at a low concentration of 50 ppm not only in endemic bacteria but also in various pathogens that could cause food poisoning, post-operative infections, nosocomial infections, and the acquisition of multidrug resistance. Therefore, it is inferred that MA-T is effective against a number of pathogens. In addition, because the BHI liquid medium used in the bacterial culture in this study contained a large amount of organic matter, MA-T is considered to be less susceptible to organic matter in its bactericidal effect. We have confirmed that MIC and MBC of hypochlorous acid are measured at around 10 ppm for *E. coli* in poor medium by using Davis minimal medium,[31] but around 600 ppm in BHI medium (Personal Communication).

The antiviral activity of MA-T against each virus was confirmed for SARS-CoV-2, IAV, SARS-CoV-1, MERS-CoV, RV-A, HCV, DENV, and HBV, and highly efficacies were obtained in particular in SARS-CoV-2, IAV, SARS-CoV-1, MERS-CoV, Hepatitis C virus, and dengue virus. On the other hand, since Feline calicivirus is a non-enveloped virus belonging to the Caliciviridae family, we assumed that it would be less effective and set the reaction time to 60 min, which resulted in a 95.30% reduction. These results suggest that MA-T shows high activity against enveloped viruses, and requires slightly higher concentration of MA-T for viruses without

Table 2. MIC and MBC in Bacteria and Fungi

Microorganisms	Gram +/- (or another property), reference (strain)	MA-T (ppm)	
		MIC	MBC
Bacillus cereus (vegetative)	Gram +, RIMD0206023	2.5	ND
Bacillus subtilis (vegetative)	Gram +, NDU157[a)]	12.5	ND
Enterococcus faecalis	Gram +, RIMD3116001	5	5
Staphylococcus aureus	Gram +, NDU101[a)]	1.56	3.12
Streptococcus mutans	Gram +, M78148[a)]	2.5	15
Streptococcus pyogenes	Gram +, β25[a)]	0.1	1
Acinetobacter baumannii	Gram -, NBRC110489	20	20
Aggregatibacter actinomycetemcomitans	Gram -, ATCC29522	35	35
Campylobacter jejuni	Gram -, RIMD366048	10	10
Corynebacterium bovis	Gram -, JCM11947	3.12	25
Corynebacterium mastitidis	Gram -, JCM12269	6.25	25
Escherichia coli	Gram -, MV1184[a)]	10	25
E. coli O157:H7	Gram -, NDU119[a)]	15	NT
Haemophilus influenzae	Gram -, RIMD0806018	3.5	5
Pasteurella multosida	Gram -, RIMD1657003	3.75	3.75
Porphyromonas gingivalis	Gram -, W83[a)]	20	20
Propionibacterium acnes	Gram -, NDU2563[a)]	0.1	NT
Pseudomonas aeruginosa	Gram -, NDU315[a)]	20	20
Salmonella Enteritidis	Gram -, RIMD1933001	2	2
Serratia marcescens	Gram -, RIMD1996001	35	45
Tannerella forsythia	Gram -, NDU2001[a)]	12.5	12.5
Vibrio parahaemolyticus	Gram -, RIMD2210001	15	15
Yersinia enterocolitica	Gram -, RIMD2501001	15	15
Yersinia pseudotuberculosis	Gram -, RIMD2503010	20	25
Treponema denticola	spirochaeta, NDU1001[a)]	25	25
Candida albicans	fungus (eukaryotic), TIMM5588	5	5
Fusarium oxysporum	fungus (eukaryotic), NBRC9469	2	NT
Thanatephorus cucumeris	fungus (eukaryotic), NBRC30939	5	10
Zygosaccharomyces rouxii	fungus (eukaryotic), NDU1993[a)]	10	10

a): Nippon Dental University, Department of Microbiology, laboratory stock
NBRC: National Institute of Technology and Evaluation
ATCC: American Type Culture Collection
RIMD: Osaka University, Research Institute for Microbial Diseases
TIMM: Teikyo University, Institute of Medical Mycology
JCM: Japan Collection of Microorganisms
ND: not determined
NT: not tested

Table 3. Efficacy Against Severe Acute Respiratory Syndrome Coronavirus 2

Test Components	Concentration (ppm)	Exposure time	Result (log $TCID_{50}$/50 μL)	Result ($TCID_{50}$/mL)	Percent reductions (%)
PBS		1 min	4.25	355656	0.00
70% EtOH		1 min	0.5	63	99.98
MA-T	50	1 min	0.5	63	99.98
	100	1 min	0.5	63	99.98
	150	1 min	0.5	63	99.98
	500	1 min	0.5	63	99.98

Table 4. Efficacy Against Other Viruses

Viruses	MA-T concentration (ppm)	Exposure time	Percent reductions (%)
Influenza A virus	100	1 min	99.99
Feline calicivirus	50	60 min	95.30
Severe acute respiratory syndrome coronavirus 1	100	1 min	98.22
Middle East respiratory syndrome coronavirus	100	1 min	99.82
Rotavirus A	200	1 min	88.9
Rotavirus A	100	1 min	33.3
Hepatitis C virus	100	1 min	99.96
Dengue virus	100	1 min	98.70
Hepatitis B virus	100	1 min	74.5

envelope such as rotavirus. For SARS-CoV-2, which is currently a global threat, MA-T showed high efficacy, and this antiviral effect was almost the same as the result of treating with 70% ethanol for 1 min.[32]

There are many types of disinfectants and germicides such as aldehydes, chlorines, alcohols, and quaternary ammoniums, and MA-T is classified as chlorine disinfectant among them. Although chlorinated agents have a reduced bactericidal activity in the presence of organic substances,[33-35] MA-T had a sufficient bactericidal effect and a broad antibacterial and antiviral spectrum at low concentrations even in the presence of organic substances. Its antibacterial and antiviral spectrum is believed to be comparable to that of other chlorine agents.[36,37] Additionally, MA-T showed high efficacy against SARS-CoV-2, MERS-CoV, SARS-CoV-1, IAV, and DENV. Therefore, MA-T can be a promising disinfectant against infections that have been prevalent in the world, including SARS-CoV-2. In MA-T, the toxicity of conventional chlorine agents, such as metal corrosion, mucous membrane irritation, and skin irritation[24,25] could not be detected. These results suggests that MA-T may be used effectively and safely against many pathogens including SARS-CoV-2. In addition, MA-T is stable in aqueous solution, does not decompose spontaneously like aqueous chlorite, acidified sodium chlorite, or hypochlorous acid,[38] and is almost neutral at pH = 7.5. Furthermore, unlike ethanol, it does not ignite or volatilize,[23] and can be stored and used after opening as long as there is no contamination, which is highly convenient. As mentioned above, MA-T is an extremely safe, effective, and convenient disinfectant, and is expected to be applied in various situations including the medical field.

There are three limitations in our study. The first is the effect on spore-forming bacteria. MA-T exhibits a bactericidal effect by the action of a radical reaction, similar to conventional chlorine-based disinfectants, but currently marketed products (A2Care®: 100 ppm MA-T, BACT-O®: 150 ppm MA-T) place importance on safety. They have high effects on enveloped viruses and bacteria that do not form spores, but are presumed to be less effective against spore-forming bacteria. Therefore, we consider that use in situations where pathogens such as Clostridium and Bacillus are of concern should be avoided. Secondly, since MA-T exhibits a bactericidal effect by exposure to pathogens like other disinfectants,[22,39] it is necessary to maintain proper usage and exposure time such as spraying, wiping, and dipping. Although there is no data for less than 1 min in this study, MA-T does not volatilize, so it does not matter as much as ethanol. The second limitation is already being overcome by modifying the catalyst to increase the equilibrium constant or developing methods to further activate the generated chlorine dioxide radicals (in preparation). In the future, it is necessary to fix the conditions for the use of MA-T for various purposes. Thirdly, MA-T has pKa = 1.9 and generates chlorine dioxide under strong acidity. However, the high acidity condition is rarely met in daily life, and the only situation it can be assumed is when an accidental ingestion occurs on an empty stomach.

The hypothesis that MA-T is a safe and effective disinfectant has been proven. MA-T is a next-generation chlorine disinfectant that combines high safety and high anti-pathogen activity to overcome the weaknesses of conventional chlorine-based disinfectants. MA-T is expected to be applied as a safer, more effective, and more convenient disinfectant in various fields where chlorine agents and ethanol are used as disinfectants. MA-T is also expected to play an important role in fields where other disinfectants have not been able to be applied due to their properties. Furthermore, it is expected that MA-T will play an important role in the prevention of SARS-CoV-2 as a disinfectant, which is as effective as ethanol in preventing SARS-CoV-2 and is less likely to volatilize.

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