Can hypochlorous acid be a powerful sanitizer to replace alcohol for disinfection ? —Its bactericidal, degradation of the solutions under various storage condition, and steel rust effects

Takashi KAMEDA¹, Shunya OKA², Jun-ichi IGAWA³, Makoto SAKAMOTO⁴ and Kazuto TERADA⁵

¹ Department of Orthodontics, Nippon Dental University School of Life Dentistry at Niigata, 1-8 Hamaura-cho, Chuo-ku, Niigata 951-8580, Japan

² Department of Biology, Nippon Dental University School of Life Dentistry at Niigata, 1-8 Hamaura-cho, Chuo-ku, Niigata 951-8580, Japan

³ Igawa Dental Office, 7-14-16 Oizumigakuen-cho, Nerima-ku, Tokyo 178-0061, Japan

⁴ Department of Health Sciences, Niigata University School of Medicine, 2-746, Asahimachi, Chuo-ku, Niigata 951-8518, Japan

⁵ Nippon Dental University Niigata Hospital, 1-8 Hamaura-cho, Chuo-ku, Niigata 951-8580, Japan

Corresponding author, Takashi KAMEDA; E-mail: tkameda_jbso@yahoo.co.jp

Coronavirus disease pandemic has led to a shortage of disinfectant alcohol (DA; 76.9–81.4% ethanol solution) in Japan. This study investigated the availability of hypochlorous acid (HA) as an alternative disinfectant to DA in clinical environment. HA is a safe and economical disinfectant with a broad antibacterial spectrum and low risk of hand irritation, which is used worldwide. In this study, HA and sodium hypochlorite solution (SH) were degraded over time and the degradation was accelerated by temperature and lighting. Results of bactericidal effects of HA revealed that the realistic effective concentration should be 200 ppm. Compared with DA, the dental carbon steel burs were more severely rusted by SH and HA. These results indicated that high concentration of HA generated on-site could be an effective and safe disinfectant for use immediately after generation with consideration of object materials in clinical environment, as an alternative to DA.

Keywords: On-site generation, Ethanol, Residual chlorine concentration, pH

INTRODUCTION

Since winter 2019 to early 2020, coronavirus disease (COVID-19) has spread worldwide and this pandemic is predicted to continue until 2022–2024¹⁾. This pandemic has caused shortage of masks and disinfectants, especially disinfectant alcohol (DA; 76.9–81.4% ethanol [EtOH] solution), in all the cities, especially medical institutions, in Japan. In addition to the prevention of droplet infection with masks, ensuring social distancing, enforcement of hand washing, and disinfection of the areas touched by the hands are important for the prevention of infection. Instead of DA, several disinfectants were shortlisted and their efficacy was investigated by an independent administrative institution, National Institute of Technology and Evaluation (NITE), at the request of the Ministry of Economy, Trade, and Industry of Japan. These NITE reports indicated the possibility of substituting sterilizing supplies with surfactants and hypochlorous acid (HA)²⁾. The US Environmental Protection Agency (EPA) recommends HA as an effective disinfectant for COVID-19³⁾, and the Center for Disease Control and Prevention (CDC) considers it a high-level disinfectant⁴⁾, and HA has been approved for clinical use^{5,6)}.

HA, which is often confused with strongly alkaline sodium hypochlorite (SH), is effective disinfectant with broad antibacterial spectrum for many bacteria with or without spore and viruses⁷⁻⁹⁾; however, careful

handling is required. Ultraviolet (UV) light and high temperature are known to degrade HA rapidly^{10,11)}. Therefore, disinfectant effects of these reagents are often highly variable and inconsistent, especially when using commercially available bottled HA with unknown information regarding their storage after manufacture¹²⁾. HA is approved as a disinfectant for food in Japan, but can only be used fresh out of the generator¹³⁾. There are three types of generators for HA: electrolysis (EL), two-liquid mixture (2Mx), and carbon dioxide mixture (CO₂) type (Fig. 1). Since the 2Mx type (mixture of SH and acidic solution as a pH regulator) may generate chlorine (Cl) gas during the HA generation process at low pH (<pH 4) in mixing stages, which can cause fatalities, EL or CO₂ type generators can be used in general facilities and medical institutions for on-site generation.

HA has strong oxidizing reagents that should be considered as one of the alternatives to DA. However, its poor shelf life and difficulty in storage could be a problem for its use. Therefore, one of the basic requirements for use of HA is to have a proper generator in possession. Hence, these reagents are not widely used presently in clinical environment.

In this study, we investigated whether HA can be used as an alternative disinfectant to DA in medical and dental practice; its disinfecting effects in the presence of organic matter, and dental carbon steel burs used in clinical field, and the degradation of reagents during storage directly related to its disinfecting effects, compared with DA.

Color figures can be viewed in the online issue, which is available at J-STAGE.

Received May 12, 2021; Accepted Jul 27, 2021

doi:10.4012/dmj.2021-146 JOI JST.JSTAGE/dmj/2021-146

Fig. 1 Three types of generation methods of hypochlorous acid. (a) Electrolysis type, (b) Two-liquid mixture type, (c) Carbon dioxide mixture type. DW; distilled water, CO₂; carbon dioxide

MATERIALS AND METHODS

Materials

We prepared SH and three types of HA as an alternative disinfectant to DA (76.9–81.4% EtOH solution, 80% was selected in this study). SH at 200 and 1,000 ppm was prepared by 300 and 60 fold-dilution of 6% SH solution (Purelox, Oyalo, Tokyo, Japan) with distilled water (DW), respectively. These are the recommended concentrations of SH¹⁴⁾: 200 ppm for wiping of usual equipment and instruments and 1,000 ppm for equipment exposed to vomit, feces, saliva, and other body fluids. The EL type HA was prepared by electrolysis of NaCl-added tap water using a generator (Fineoxer FOW-1000S6-D/G, First Ocean, Kanagawa, Japan) at on-site. The 2Mx type HA was commercially available in bottled products (Product A; Cl concentration: not mentioned,

pH: weak acidity, pH regulator; CH₃COOH, Product B; Cl concentration: 50 ppm, pH 6.5, pH regulator; HCl, Product C; Cl concentration: 100 ppm, pH 5.0–6.5, pH regulator, Product D; Cl concentration: 100 ppm, pH 6.5, pH regulator; HCl, Product E; Cl concentration: 200 ppm, pH 6.5, pH regulator; HCl. Bottled products A–D were purchased through online site. Bottled product E was directly purchased and shipped to us in a refrigerated state immediately after generation, directly from the manufacturing plant). The CO₂ type HA of 120 ppm was carbonated SH diluted with tap water using a generator (KHM-1, Renafine, Tokyo, Japan). High concentration (200 ppm) of CO₂ type HA was obtained from our newly developed prototype generator. Tap water of the Nippon Dental University at Niigata was used in this study (pH: 5.7–6.2, free Cl concentration: 0.4±0.15 ppm) and was filtered to remove small contaminants with a filter

Fig. 2 Experimental flow.

DW; distilled water, DA; 80% EtOH solution, SH; sodium hypochlorite, HA; hypochlorous acid, NRS; normal rabbit serum

(Plastic filter housing with cartridge filter, Kankyo Technos, Wakayama, Japan), which was fed into the generator to produce HA (Fig. 2). Further, 80% EtOH solution as DA was diluted to 99.5% EtOH (Hayashi Pure Chemical Industries, Osaka, Japan) with DW. Carbon steel burs for contra-angle dental handpieces (ELA steel bur CA 6, Shofu, Tokyo, Japan) were used for rust test of dental carbon steel burs.

Specification of bottled HA

Prior to following experiments, we checked the pH and residual Cl concentration (RCC) (Fig. 3) of bottled 2Mx type HA (SH and HCl or CH₃COOH) with pH meter (LAQUAtwin B-71X, Horiba, Kyoto, Japan) and chlorometer (HI96771, Hanna Instruments Japan, Chiba, Japan; measurement accuracy: RCC: 1.0 ppm) respectively, to check the matches to the catalog

specifications of products instructed.

Storage condition-related degradation of disinfectants

SH, three types of HA, and 80% EtOH solution as DA were stored in tightly sealed clear plastic centrifugal tube (15 mL solution in 15 mL centrifuge tube; tube material: polypropylene; cap material: polyethylene, BM Equipment, Tokyo, Japan) under the following three conditions: storage at room temperature (RT: 22.5±0.83°C) with fluorescent lamp lighting (LT; illumination: 2022.5±84.01 lx and UV intensity: 10.7±1.21 µW/cm²), storage at RT with shading (Shd), and storage at 4°C with Shd (in a refrigerator [4C]). The plastic tube containing the solution was covered entirely with aluminum foil and placed in a lightproof box for Shd. Illumination and UV intensity were estimated by an illuminometer (LX2; Sanwa Electric Instrument, Tokyo,

Fig. 3 State of hypochlorous acid.

2Mx; Two-liquid mixture type, EL; Electrolysis type, CO_2 ; Carbon dioxide mixture type, SH; sodium hypochlorite, HA; hypochlorous acid. Blue dotted arrows indicate pH of bottled commercial 2Mx HA used in specification check. Red solid arrows indicate pH of HAs used in degradation, bactericidal, and rust tests.

Japan) and UV intensity meter (SP-82UV, Mother Tool, Tochigi, Japan), respectively. The EL type and CO_2 type HA produced by each generator at on-site were used at the concentration as they came out of each generator. Unadjusted concentrations of the solutions were used because matching the concentrations of each solution could have caused the disinfectant effect and pH to work improperly (Fig. 3). Measurement of pH for all solutions with a pH meter; RCC for SH, each HA, and tap water with a chlorometer; and EtOH concentration with an EtOH concentration meter (PAL-corona disinfectant checker, Atago, Tokyo, Japan; measurement accuracy: $\pm 1.0\%$), were performed at, before, and each storage point: 0, 1, 3, 7 (1 week), 14 (2 weeks), 21 (3 weeks), and 30 days (1 month) (Fig. 2). Since EtOH is an organic solvent, pH measurement was not performed, as it is measurable. During storage, the bottle of each solution was opened and exposed to air for 1 min a day.

Bactericidal effects of disinfectants under the condition with organic matter

For evaluation of the bactericidal effects of disinfectants, we prepared typical oral bacteria: *Streptococcus mutans* (JCM 5705, the RIKEN BioResource Center via the National BioResource Project of MEXT, Ibaraki, Japan) and nosocomial infection-related bacteria: *Staphylococcus aureus* (JCM2151, the RIKEN BioResource Center) and *Escherichia. coli* (JCM1649T, the RIKEN BioResource Center). These bacteria were cultured in highly nutritious medium, i.e., brain heart infusion (BHI) medium (Beckton, Dickinson, Franklin Lakes, NJ, USA) at 37°C . This bactericidal test was performed according to ASTM E2315-03 1 Time-Kill test (ASTM E2315-03 Standard Guide for Assessment of Antimicrobial Activity Using a Time-Kill Procedure) (Fig. 2).

Bacteria solution (500 μL BHI medium of OD 0.5 solution, which was subtracted by OD value of BHI medium only) was poured to each disinfectant solution (9.5 mL), mixed for 10 s, and placed for 0, 20,

and 50 s (total treatment time: 10, 30, and 60 s). After disinfectant treatment, 0.1 mL of bacteria solution with each disinfectant was poured to 9.9 mL of BHI medium, and mixed for 10 s. Further, 20 μ L of each mixed solution was disseminated on an agar plate (Agar, powder; 010-15815 Fujifilm Wako Pure Chemical, Osaka, Japan), cultured at 37°C overnight, and the colony number was estimated. In the experimental condition without organic matter, disinfectants were created as 9 mL of each disinfectant solution with 0.5 mL of DW (total 9.5 mL), and then bacteria solution was added in 0.5 mL BHI medium. In the experimental condition with organic matter, disinfectants were created as 9 mL of each disinfectant solution, and then 0.5 mL bacteria solution was added with 0.5 mL of normal rabbit serum (NRS; ICN Biomedicals, Aurora, OH, USA) solution in DW, which indicated that final concentration of NRS was 5%. Final concentration of each disinfectant containing 0.5 mL of bacteria solution with 0.5 mL of NRS or DW (total 10 mL) was 90% concentration of added disinfectant (Fig. 2). Control group was added DW instead of disinfectant. Bactericidal effects were assessed as a percentage of colony number of each bacterium with DW treatment (control group).

Rust test of dental steel burs after disinfectant repeated-exposure

Rust of dental carbon steel burs for contra-angle dental handpieces was accessed by visual and microscopic observation under a stereoscopic microscope (SMZ800, Nikon, Tokyo, Japan), the images were captured into the computers by CCD camera (ARTCAM-130MI, Artray, Tokyo, Japan) (Fig. 2). Dental carbon steel burs were immersed in 3 mL of disinfectants in tightly sealed 3

mL vials. After dental burs were immersed in SH and HA for 15 min, they were washed with DW twice; water droplets were blown with air, and air-dried for 30 min. These immersion-air-dry procedures were repeated eight times (total disinfectant immersion time: 2 h).

Experimental conditions and data and statistical analysis

All experiments were performed in a laboratory environment maintained at 22–24°C (RT) with humidity of 40–50% under the room luminance of 400–500 lx with UV intensity of below measurable value of the UV meter. Each experiment was repeated 10 times independently, with the maximum and minimum values in each data set being removed before calculation of mean values. Data are presented as the mean $\pm$ standard deviation. The statistical significance of the differences within storage periods and storage conditions in same disinfectants was determined with the Tukey-Kramer test. Statistical significance was set at $p < 0.01$. Statistical analysis was performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing; Vienna University of Technology, Vienna, Austria) on a workstation computer (MB-P5300X-WS, Mouse Computer, Tokyo, Japan).

RESULT

Specifications for bottled commercial HA

Prior to storage condition-related degradation test, we checked the pH and RCC (total Cl concentration) of five bottled HAs available in the market (Table 1). The measured specifications of two out of the five products did

Table 1 Chlorine concentration and pH of commercially available bottled hypochlorous acid produced by mixing sodium hypochlorite and pH regulators

Product	Nominal value /data			Measured value			
	Cl (ppm)	pH	pH regulator	RCC (ppm)		pH	
				Value	AR	Value	Dev
A	no	no	CH ₃ COOH	87.7 (1.87)	n/a	4.98 (0.08)	n/a
B	50	6.5	HCl	38.2 (1.17)	76.4% (2.34)	6.54 (0.07)	+0.04
C	100	5.0–6.5	HCl	117.7 (1.97)	117.7% (1.97)	3.36 (0.04)	–3.14~–1.64
D	100	6.5	HCl	6.83 (0.63)	6.83% (0.63)	3.27 (0.03)	–3.23
E	200	6.5	HCl	207.8 (4.49)	103.9% (2.25)	6.68 (0.04)	+0.18

Values are represented as mean and (SD).

Cl; chlorine, RCC; residual chlorine concentration

no; no data in catalog, bottle label and home page of a product, n/a; not available, AR; achievement rate, Dev; deviation

Measurements were averaged over eight measurements taken every 15 min of same bottled HA.

Table 2 Degradation of disinfectants during various storage conditions

A. Sodium hypochlorite

a) 200 ppm

Storage condition	Measurement item	Storage period (day)							
		0	1	3	7	14	21	30	
4C	RCC	Raw data	201.2 ^a (5.21)	200.5 ^{aA} (3.35)	198.1 ^{aA} (3.11)	197.3 ^{aA} (4.55)	196.6 ^{aA} (3.38)	195.8 ^{aA} (4.07)	195.1 ^{aA} (4.11)
		% of day0	100.0 (2.59)	99.7 (1.67)	98.5 (1.55)	98.1 (2.26)	97.7 (1.68)	97.3 (2.02)	97.0 (2.04)
	pH	9.58 ^a (0.14)	9.48 ^{abA} (0.13)	9.42 ^{abA} (0.11)	9.36 ^{bcA} (0.14)	9.18 ^{cdA} (0.16)	8.94 ^{deA} (0.18)	8.83 ^{eA} (0.16)	
RT-Shd	RCC	Raw data	↑	199.7 ^{aA} (3.94)	196.6 ^{abA} (3.77)	193.4 ^{bcA} (4.12)	191.9 ^{bcA} (4.22)	191.1 ^{bcA} (4.65)	188.8 ^{cB} (3.56)
		% of day0	↑	99.3 (1.96)	97.7 (1.87)	96.2 (2.05)	95.4 (2.10)	95.0 (2.31)	93.8 (1.77)
	pH	↑	9.56 ^{aA} (0.16)	9.54 ^{aA} (0.14)	9.39 ^{aA} (0.18)	9.09 ^{abAB} (0.15)	8.73 ^{cA} (0.14)	8.68 ^{cA} (0.13)	
RT-LT	RCC	Raw data	↑	198.9 ^{abA} (4.22)	193.4 ^{ba} (4.13)	157.6 ^{cB} (3.98)	103.0 ^{dB} (3.76)	65.4 ^{eB} (3.12)	43.7 ^{fC} (1.38)
		% of day0	↑	98.9 (2.10)	96.1 (2.05)	78.3 (1.98)	51.2 (1.87)	32.5 (1.55)	21.7 (0.69)
	pH	↑	9.53 ^{abA} (0.13)	9.48 ^{abA} (0.12)	9.32 ^{ba} (0.17)	8.91 ^{cB} (0.16)	8.26 ^{dB} (0.14)	7.73 ^{eB} (0.19)	

b) 1,000 ppm

Storage condition	Measurement item	Storage period (day)							
		0	1	3	7	14	21	30	
4C	RCC	Raw data	994.5 ^a (17.01)	992.1 ^{aA} (12.94)	984.8 ^{abA} (14.73)	977.8 ^{abA} (14.67)	968.6 ^{ba} (13.41)	943.2 ^{cA} (10.21)	921.5 ^{dA} (12.12)
		% of day0	100.0 (1.71)	99.8 (1.30)	99.0 (1.48)	98.3 (1.48)	97.4 (1.35)	94.8 (1.03)	92.7 (1.22)
	pH	10.47 ^a (0.12)	10.48 ^{aA} (0.14)	10.49 ^{aA} (0.08)	10.50 ^A (0.16)	10.51 ^{aA} (0.18)	10.57 ^{aA} (0.16)	10.62 ^{aA} (0.15)	
RT-Shd	RCC	Raw data	↑	980.7 ^{aA} (15.28)	973.5 ^{abA} (16.33)	955.3 ^{bcB} (15.32)	935.7 ^{cB} (15.01)	902.3 ^{dB} (11.18)	868.3 ^{eB} (13.48)
		% of day0	↑	98.6 (1.54)	97.9 (1.64)	96.1 (1.54)	94.1 (1.51)	90.7 (1.12)	87.3 (1.36)
	pH	↑	10.51 ^{aA} (0.17)	10.52 ^{aA} (0.16)	10.53 ^{aA} (0.17)	10.54 ^{aA} (0.15)	10.53 ^{aA} (0.16)	10.52 ^{aA} (0.14)	
RT-LT	RCC	Raw data	↑	971.0 ^{aA} (16.23)	886.8 ^{bbB} (11.60)	765.5 ^{cC} (12.30)	452.8 ^{dC} (17.54)	255.3 ^{eC} (10.86)	46.7 ^{fC} (2.17)
		% of day0	↑	97.6 (1.63)	89.2 (1.17)	77.0 (1.24)	46.6 (1.76)	26.3 (1.09)	4.70 (0.22)
	pH	↑	10.52 ^{aA} (0.13)	10.53 ^{aA} (0.11)	10.56 ^{aA} (0.19)	10.48 ^{aA} (0.17)	10.18 ^{bbB} (0.17)	9.22 ^{cB} (0.21)	

Table 2 continued

B. Hypochlorous acid

a) Bottled product - mixture of sodium hypochlorite and HCl

Storage condition	Measurement item	Storage period (day)							
		0	1	3	7	14	21	30	
4C	RCC	Raw data	207.8 ^a (4.49)	203.8 ^{abA} (7.21)	198.9 ^{bcA} (6.92)	193.1 ^{cdA} (6.23)	186.7 ^{dA} (6.66)	184.3 ^{dA} (6.98)	183.7 ^{dA} (7.41)
		% of day0	100.0 (2.16)	98.1 (3.47)	95.7 (3.33)	92.9 (3.00)	89.8 (3.21)	88.7 (3.36)	88.4 (3.57)
	pH	6.68 ^a (0.14)	6.64 ^{aA} (0.15)	6.62 ^{aA} (0.15)	6.59 ^{aA} (0.14)	6.57 ^{aA} (0.21)	6.55 ^{aA} (0.17)	6.52 ^{aA} (0.16)	
RT-Shd	RCC	Raw data	↑	200.3 ^{abA} (6.88)	191.3 ^{bcAB} (6.51)	184.1 ^{cA} (7.01)	172.8 ^{dB} (6.87)	169.4 ^{dB} (7.41)	164.3 ^{dB} (6.38)
		% of day0	↑	96.4 (3.31)	92.1 (3.11)	88.6 (3.37)	83.2 (3.31)	81.5 (3.57)	79.1 (3.07)
	pH	↑	6.57 ^{abAB} (0.14)	6.53 ^{abAB} (0.15)	6.47 ^{abAB} (0.16)	6.43 ^{ba} (0.15)	6.41 ^{ba} (0.12)	6.38 ^{ba} (0.09)	
RT-LT	RCC	Raw data	↑	196.2 ^{aA} (7.38)	181.8 ^{bB} (7.98)	166.2 ^{cB} (6.98)	138.5 ^{dC} (7.21)	104.3 ^{eC} (7.33)	51.3 ^{fC} (6.65)
		% of day0	↑	94.4 (3.55)	87.5 (3.84)	80.0 (3.36)	66.7 (3.47)	50.2 (3.53)	24.7 (3.20)
	pH	↑	6.39 ^{bB} (0.13)	6.38 ^{bB} (0.15)	6.27 ^{bB} (0.19)	5.86 ^{cB} (0.14)	4.83 ^{dB} (0.13)	3.15 ^{eB} (0.11)	

b) Electrolysis type at on-site generation

Storage condition	Measurement item	Storage period (day)							
		0	1	3	7	14	21	30	
4C	RCC	Raw data	54.7 ^a (1.51)	54.0 ^{abA} (1.41)	52.7 ^{ba} (0.82)	49.5 ^{cA} (1.76)	43.3 ^{dA} (2.27)	39.8 ^{eA} (2.12)	36.5 ^{fA} (1.38)
		% of day0	100.0 (2.76)	98.7 (2.58)	96.3 (1.50)	90.5 (3.22)	79.2 (4.15)	72.8 (3.88)	66.7 (2.52)
	pH	3.31 ^a (0.16)	3.31 ^{aA} (0.16)	3.30 ^{aA} (0.15)	3.24 ^{aA} (0.17)	3.22 ^{aA} (0.16)	3.21 ^{aA} (0.09)	3.19 ^{aA} (0.14)	
RT-Shd	RCC	Raw data	↑	51.3 ^{bB} (1.21)	45.3 ^{cB} (2.16)	39.2 ^{dB} (0.74)	32.2 ^{eB} (1.86)	27.8 ^{fB} (1.65)	22.0 ^{gB} (1.79)
		% of day0	↑	93.8 (2.21)	82.8 (3.95)	71.7 (1.35)	58.9 (3.40)	50.8 (3.02)	40.2 (3.27)
	pH	↑	3.31 ^{aA} (0.13)	3.26 ^{aA} (0.13)	3.22 ^{aA} (0.15)	3.14 ^{aA} (0.14)	3.13 ^{aAB} (0.11)	3.10 ^{aAB} (0.15)	
RT-LT	RCC	Raw data	↑	47.7 ^{bC} (1.03)	40.8 ^{cC} (2.16)	27.6 ^{dC} (1.26)	17.5 ^{eC} (1.98)	9.3 ^{fC} (0.89)	1.1 ^{gC} (0.16)
		% of day0	↑	87.2 (1.88)	74.6 (3.95)	50.5 (2.30)	32.0 (3.62)	17.0 (0.43)	2.0 (0.29)
	pH	↑	3.30 ^{aA} (0.15)	3.21 ^{abA} (0.13)	3.12 ^{abcA} (0.14)	3.01 ^{bcA} (0.16)	2.95 ^{cB} (0.18)	2.92 ^{cB} (0.16)	

Table 2 continued

c) CO₂ mixture type at on-site generation

(1) 120 ppm by a product generator

Storage condition	Measurement item	Storage period (day)							
		0	1	3	7	14	21	30	
4C	RCC	Raw data	127.6 ^a (3.49)	126.2 ^{aA} (2.30)	121.2 ^{ba} (2.05)	116.9 ^{ca} (3.05)	113.2 ^{cdA} (2.16)	109.6 ^{dA} (2.77)	105.5 ^{ea} (2.65)
		% of day0	100.0 (2.74)	98.9 (1.80)	95.0 (1.61)	91.6 (2.39)	88.7 (1.69)	85.9 (2.17)	82.6 (2.08)
	pH	6.50 ^a (0.15)	6.50 ^{aA} (0.14)	6.52 ^{aA} (0.13)	6.55 ^{aA} (0.13)	6.57 ^{aA} (0.09)	6.56 ^{aA} (0.16)	6.56 ^{aA} (0.15)	
RT-Shd	RCC	Raw data	↑	124.8 ^{aA} (2.93)	117.0 ^{bb} (2.61)	107.7 ^{cb} (1.86)	96.5 ^{dB} (2.67)	87.7 ^{eb} (3.10)	80.3 ^{fb} (2.48)
		% of day0	↑	97.8 (2.30)	91.7 (2.05)	84.4 (1.46)	75.6 (2.09)	68.7 (2.43)	62.9 (1.94)
	pH	↑	6.51 ^{aA} (0.12)	6.53 ^{abA} (0.14)	6.59 ^{abA} (0.12)	6.68 ^{abA} (0.16)	6.79 ^{bcB} (0.11)	6.94 ^{cb} (0.14)	
RT-LT	RCC	Raw data	↑	116.5 ^{bb} (3.08)	107.3 ^{cc} (3.25)	88.5 ^{dc} (3.37)	62.1 ^{ec} (3.82)	36.4 ^{fc} (3.21)	11.2 ^{gc} (1.97)
		% of day0	↑	91.3 (2.41)	84.1 (2.55)	69.4 (2.64)	48.7 (2.99)	28.5 (2.52)	8.8 (1.54)
	pH	↑	6.53 ^{aA} (0.12)	6.59 ^{aA} (0.17)	6.65 ^{aA} (0.14)	6.72 ^{aA} (0.15)	6.68 ^{aAB} (0.19)	6.63 ^{aA} (0.14)	
(2) 200 ppm by a prototype generator									
Storage condition	Measurement item	Storage period (day)							
		0	1	3	7	14	21	30	
4C	RCC	Raw data	240.8 ^a (6.43)	237.2 ^a (3.52)	227.8 ^b (3.44)	217.7 ^c (4.50)	208.1 ^d (6.55)	197.7 ^e (5.63)	189.8 ^f (4.09)
		% of day0	100.0 (2.67)	98.5 (1.46)	94.6 (1.43)	90.4 (1.87)	86.4 (2.72)	82.1 (2.34)	78.8 (1.70)
	pH	6.21 ^a (0.16)	6.22 ^{aA} (0.09)	6.23 ^{aA} (0.17)	6.25 ^{aA} (0.11)	6.31 ^{aA} (0.13)	6.34 ^{aA} (0.13)	6.33 ^{aA} (0.09)	
RT-Shd	RCC	Raw data	↑	221.2 ^b (5.06)	212.7 ^c (4.10)	197.3 ^d (5.61)	169.8 ^e (5.13)	147.3 ^f (6.58)	124.5 ^g (4.49)
		% of day0	↑	91.9 (2.10)	88.3 (1.70)	81.9 (2.33)	70.5 (2.13)	61.2 (2.73)	51.7 (1.86)
	pH	↑	6.23 ^{aA} (0.15)	6.24 ^{aA} (0.14)	6.28 ^{aA} (0.16)	6.37 ^{abAB} (0.15)	6.53 ^{bcA} (0.13)	6.69 ^{cb} (0.17)	
RT-LT	RCC	Raw data	↑	210.5 ^b (4.85)	198.2 ^c (5.64)	165.7 ^d (4.88)	111.2 ^e (4.36)	48.9 ^f (4.05)	5.4 ^g (1.38)
		% of day0	↑	87.4 (2.01)	82.3 (2.34)	68.8 (2.03)	46.2 (1.81)	20.3 (1.68)	2.2 (0.57)
	pH	↑	6.25 ^{aA} (0.14)	6.31 ^{abA} (0.16)	6.35 ^{abA} (0.17)	6.43 ^{abB} (0.15)	6.47 ^{ba} (0.11)	6.46 ^{ba} (0.12)	

Table 2 continued

C. Disinfectant alcohol (80% ethanol solution)

Storage condition	Measurement item	Storage period (day)							
		0	1	3	7	14	21	30	
4C	EtOH	Raw data	80.9 ^a (1.06)	80.2 ^{abA} (1.26)	79.8 ^{abA} (0.92)	79.4 ^{abcA} (0.99)	79.1 ^{bcA} (1.02)	78.6 ^{bcA} (0.89)	78.1 ^{cA} (1.09)
		% of day0	100.0 (1.31)	99.1 (1.56)	98.6 (1.14)	98.1 (1.22)	97.8 (1.26)	97.2 (1.10)	96.5 (1.35)
RT-Shd	EtOH	Raw data	↑	79.6 ^{abA} (1.16)	79.2 ^{ba} (1.08)	78.6 ^{bcA} (1.11)	78.3 ^{bcA} (0.84)	77.1 ^{cB} (0.96)	75.4 ^{dB} (0.89)
		% of day0	↑	98.4 (1.43)	97.9 (1.33)	97.2 (1.37)	96.8 (1.04)	95.3 (1.19)	93.2 (1.10)
RT-LT	EtOH	Raw data	↑	78.7 ^{ba} (1.04)	77.5 ^{bcB} (1.25)	76.7 ^{cB} (1.17)	75.9 ^{cB} (0.91)	73.8 ^{dC} (0.95)	70.2 ^{cC} (0.99)
		% of day0	↑	97.3 (1.29)	95.8 (1.55)	94.8 (1.45)	93.8 (1.12)	91.2 (1.17)	86.8 (1.22)

$n=8$ for each experimental condition.

Values are represented as mean and (SD). HA; hypochlorous acid, RCC; residual chlorine concentration, EtOH; ethanol, 4C; 4°C with shading in a refrigerator, RT; room temperature, Shd; shading, LT; lighting.

Temperature: $22.5 \pm 0.83^\circ\text{C}$, illumination (fluorescent lamp lighting): 2022.5 ± 84.01 lx, and UV intensity: $10.7 \pm 1.21 \mu\text{W}/\text{cm}^2$.

Different superscript small and capital letters denote statistically significant differences ($p < 0.01$)

within the storage periods containing day 0 under the same storage condition, and within the storage conditions in the same storage periods, respectively.

not meet the product's published specifications (product B and D in Table 1). Product A had no specifications listed and the RCC of product C was almost 20% over the listed specifications. Only product E satisfied the nominal value that was shipped in a refrigerated state immediately after generation.

From these results, we investigated HA generated at laboratory using two kinds of generators: EL and CO₂ types, in subsequent experiments with bottled product E (mixture of SH and HCl), SH, and DA (80% EtOH solution).

Storage condition-related degradation test of disinfectants

To examine the degradation of the solution's potency during storage, RCC and pH in SH and HA, and EtOH concentration in DA were measured over time (Tables 2A–C). We estimated free Cl concentration of each solution at the same time of RCC estimation, and these values were almost similar to RCC (data not shown). The RCC and pH of each solution was measured after storage at RT under LT of fluorescent lamps for 24 h per day, RT-storage with Shd, and storage at 4°C in a refrigerator with Shd (4C) for 0 (immediate after generation), 1, 3, 7 (1 week), 14 (2 weeks), 21 (3 weeks), and 30 days (1 month).

As for the SH solution (Tables 2A a), b), and 3), RCC did not decrease significantly in 30 days for both the 4C and RT-Shd-storage solutions. In contrast, RCC of the specimens exposed to LT at RT decreased linearly with time, and decreased by half in 14 days. On day 30, RCC

decreased by approximately 95% in 1,000 ppm and 80% in 200 ppm. The decrease in pH was accompanied with the decrease in RCC.

The changes in RCC and pH were also studied for three types of four HAs: a bottled HA, an EL type HA generated at on-site, and two concentrations of CO₂ HAs generated at on-site (Tables 2B and 3). For bottled HA produced by mixing SH and HCl, RCC in Shd groups (4C and RT-Shd) decreased slowly with constant pH over time. In contrast, RCC of the specimens exposed to LT at RT decreased linearly with time, and decrease in pH was accompanied with the decrease in RCC (Tables 2B a) and 3). These changes were similar to those of SH solution. In contrast, the RCC of EL type HA decreased rapidly even in 4C storage. Furthermore, RCC decreased equally with time by the effects of temperature and lighting (Tables 2B b) and 3). RCC seemed to drop rapidly during the first week and then linearly. It decreased approximately two-third of Cl concentration in 30 days for the 4C ones, one-third at RT-Shd, and almost zero for the ones exposed to LT at RT. There was no significant change in pH in the EL type HA. The CO₂ type HAs generated from both the generators in the market (nominal value: 120 ppm, measured RCC: 127.6 ppm) and prototype (measured RCC: 240.8 ppm) showed essentially the same decrease pattern in RCC and pH (Tables 2B c) (1) (2) and 3). The decrease in RCC by temperature was more pronounced at higher concentrations; however, the effect of lighting was greater than that of temperature. In the CO₂ type HA, unlike the SH and 2Mx type bottled HA, the pH increased with degradation. The decrease in RCC over

time was linear, decreasing by approximately 10% and 5% in the 4C samples and 20% and 10% in the RT-Shd samples of 120 ppm and 200 ppm after 30 day-storage, respectively. RCC of 120 ppm and 200 ppm HA exposed to lighting at RT decreased linearly to 1/10 and 1/50 in

30 days.

For DA, *i.e.*, 80% EtOH (Tables 2C and 3), the concentration of the 4C-EtOH was almost unchanged in 30 days; however, the concentration decreased after 3 weeks and decreased by approximately 7% after 30 day-

Table 3 Comparison of residual chlorine concentration changes of different method generated hypochlorous acid under various storage conditions

A. Storage at 4°C with shading in a refrigerator

% of RCC at day 0		Storage periods (day)						
		0	1	3	7	14	21	30
Bottled product mixture of SH and HCl		100.0 ^A (2.16)	98.1 ^A (3.47)	95.7 ^A (3.33)	92.9 ^A (3.00)	89.8 ^A (3.21)	88.7 ^A (3.36)	88.4 ^A (3.57)
Electrolysis type at on-site generation		100.0 ^A (2.76)	98.7 ^A (2.58)	96.3 ^A (1.50)	90.5 ^A (3.22)	79.2 ^B (4.15)	72.8 ^C (3.88)	66.7 ^D (2.52)
CO ₂ mixture type at on-site generation	120 ppm	100.0 ^A (2.74)	98.9 ^A (1.80)	95.0 ^A (1.61)	91.6 ^A (2.39)	88.7 ^A (1.69)	85.9 ^A (2.17)	82.6 ^B (2.08)
	200 ppm	100.0 ^A (2.67)	98.5 ^A (1.46)	94.6 ^A (1.43)	90.4 ^A (1.87)	86.4 ^A (2.72)	82.1 ^B (2.34)	78.8 ^C (1.70)

B. Storage at room temperature with shading

% of RCC at day 0		Storage periods (day)						
		0	1	3	7	14	21	30
Bottled product mixture of SH and HCl		100.0 ^A (2.16)	96.4 ^{AB} (3.31)	92.1 ^A (3.11)	88.6 ^A (3.37)	83.2 ^A (3.31)	81.5 ^A (3.57)	79.1 ^A (3.07)
Electrolysis type at on-site generation		100.0 ^A (2.76)	93.8 ^{BC} (2.21)	82.8 ^C (3.95)	71.7 ^C (1.35)	58.9 ^D (3.40)	50.8 ^D (3.02)	40.2 ^D (3.27)
CO ₂ mixture type at on-site generation	120 ppm	100.0 ^A (2.74)	97.8 ^A (2.30)	91.7 ^A (2.05)	84.4 ^B (1.46)	75.6 ^B (2.09)	68.7 ^B (2.43)	62.9 ^B (1.94)
	200 ppm	100.0 ^A (2.67)	91.9 ^C (2.10)	88.3 ^B (1.70)	81.9 ^B (2.33)	70.5 ^C (2.13)	61.2 ^C (2.73)	51.7 ^C (1.86)

C. Storage at room temperature with lighting

% of RCC at day 0		Storage periods (day)						
		0	1	3	7	14	21	30
Bottled product mixture of SH and HCl		100.0 ^A (2.16)	94.4 ^A (3.55)	87.5 ^A (3.84)	80.0 ^A (3.36)	66.7 ^A (3.47)	50.2 ^A (3.53)	24.7 ^A (3.20)
Electrolysis type at on-site generation		100.0 ^A (2.76)	87.2 ^B (1.88)	74.6 ^C (3.95)	50.5 ^C (2.30)	32.0 ^C (3.62)	17.0 ^D (0.43)	2.0 ^C (0.29)
CO ₂ mixture type at on-site generation	120 ppm	100.0 ^A (2.74)	91.3 ^A (2.41)	84.1 ^{AB} (2.55)	69.4 ^B (2.64)	48.7 ^B (2.99)	28.5 ^B (2.52)	8.8 ^B (1.54)
	200 ppm	100.0 ^A (2.67)	87.4 ^B (2.01)	82.3 ^B (2.34)	68.8 ^B (2.03)	46.2 ^B (1.81)	20.3 ^C (1.68)	2.2 ^C (0.57)

n=8 for each experimental condition.

Values are represented as percentage (%) of day 0 residual chlorine concentration, and mean and (SD).

RCC; residual chlorine concentration, SH; sodium hypochlorite.

Temperature: 22.5±0.83°C, illumination (fluorescent lamp lighting): 2022.5±84.01 lx, and UV intensity: 10.7±1.21 µW/cm².

Superscript letters denote statistically significant differences (*p*<0.01) within the same storage periods under the same storage condition of different HAs.

Table 4 continued
B. *Streptococcus mutans*

Disinfectants	Concentration nominal value (measured value)	Treatment time					
		30 s		60 s			
		Bacteria in 5% BHI medium with NRS of					
		0%	5%	0%	5%		
Distilled water	— (pH6.32)	Cont	Cont	Cont	Cont		
		100.0	100.0	100.0	100.0		
		(8.89)	(7.56)	(8.36)	(4.96)		
SH solution	200 ppm (202.1 ppm, pH9.53)	+++	+++	+++	+++		
		0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)		
	1,000 ppm (993.2 ppm, pH10.45)	+++	+++	+++	+++		
		0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)		
	Bottled product (SH and HCl mixture)	200 ppm (207.6 ppm, pH6.72)	+++	+++	+++	+++	
			0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	
Hypochlorous acid On-site generation	Electrolysis type	—	—	—	—		
		20–60 ppm (55.3 ppm, pH3.21)	8.92 (1.05)	17.3 (1.70)	2.11 (0.47)	8.81 (0.94)	
	CO ₂ mixture type	120 ppm (127.2 ppm, pH6.54)	± 0.71 (0.53)	— 7.61 (0.52)	+++ 0.00 (0.00)	+ 0.47 (0.07)	
		200 ppm (240.4 ppm, pH6.23)	+++ 0.00 (0.00)	+++ 0.00 (0.00)	+++ 0.00 (0.00)	+++ 0.00 (0.00)	
		Ethanol	80% (80.6%)	+++	+++	+++	+++
				0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)

effect of SH (1,000 ppm), CO₂ type HA (200 ppm), and DA was investigated using *E.coli*, which showed the most stable effects (Table 4). The results showed that all disinfectants were completely disinfected without NRS; however, colony formation of *E. coli* was observed approximately 0.14% in SH and 0.18% in HA in cases with the addition of 5% NRS. No colony formation was observed in DA, even in the presence of 5% NRS.

Rust of dental steel burs after repeated-disinfectant exposure

Rust on dental steel burs is commonly seen in clinical environment. The effect of the disinfectant on the rusting of steel burs was investigated (Fig. 4). The results showed that rust on the surface of steel burs occurred even in both DW and 80% EtOH, and the degree of rust was almost the same. The rust of 50 ppm

EL type HA and 120 ppm CO₂ type HA treated dental burs was observed as the same level; however, 200 ppm CO₂ type HA-treatment produced more rust on dental burs than 50 ppm EL type HA and 120 ppm CO₂ type HA-treatment. SH produced an even greater amount of rust compared to that of HA group. In DW, DA, and HA, the rust developed on the surface of dental steel burs; however, SH penetrated more deeply (Fig. 4).

DISCUSSION

The medical profession is a high-risk occupation for various infections, including COVID-19^{15,16}. Disinfectants are important for prevention of infection, and the most widely used one in Japan is DA containing 76.9–81.4% (15°C) of EtOH. There has been a shortage of DA in Japan due to COVID-19. Therefore, surfactants,

Table 4 continued
C. Staphylococcus aureus

Disinfectants	Concentration nominal value (measured value)	Treatment time				
		30 s		60 s		
		Bacteria in 5% BHI medium with NRS of				
		0%	5%	0%	5%	
Distilled water	— (pH6.31)	Cont	Cont	Cont	Cont	
		100.0	100.0	100.0	100.0	
		(9.05)	(6.32)	(8.47)	(6.89)	
SH solution	200 ppm (201.7 ppm, pH9.51)	+++	+++	+++	+++	
		0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	
	1,000 ppm (992.4 ppm, pH10.51)	+++	+++	+++	+++	
		0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	
	Bottled product (SH and HCl mixture)	200 ppm (205.8 ppm, pH6.69)	+++	+++	+++	+++
			0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Hypochlorous acid On-site generation	Electrolysis type	—	—	—	—	
		11.1 (0.82)	22.3 (2.01)	7.68 (0.46)	14.9 (1.71)	
	CO ₂ mixture type	—	—	+++	±	
		2.44 (0.27)	8.88 (1.34)	0.00 (0.00)	0.67 (0.14)	
		+++	+++	+++	+++	
		0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	
Ethanol	80% (80.9%)	+++	+++	+++	+++	
		0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	

n=8 for each experimental condition.

Values are represented as mean(% of each distilled water treatment group) and (SD).

SH; sodium hypochlorite, CO₂; carbon dioxide, BHI; brain heart infusion medium, NRS; normal rabbit serum, ND; not done, Cont; control

Bactericidal level (value %; colony formation) 0%=+++; 0<+≤0.1%, 0.1<+≤0.5%, 0.5<±≤1.0%, 1%<-.

HA, and SH are suggested as its alternatives²⁾. HA is used as a disinfectant worldwide, and the US EPA and CDC considers it a high-level disinfectant^{3,4)}. Moreover, HA is now recognized as a widely utilized agent for the management of blepharitis in the United States^{5,6)}. This simple chemical compound has a broad spectrum of activity and exhibits rapid kill kinetics against a wide range of bacterial and viral organisms⁶⁾. Ophthalmic formulations of HA are safe and extremely well tolerated by the patients. In this study, we focused on HA, which has a strong oxidation capacity with high safety, to

investigate the bactericidal effects, and the degradation of the solution during various storage conditions to know how to use and store it correctly, and steel rust with disinfectant exposure, which were compared to those of DA.

DA is easy to obtain, and generally considered to have a good shelf life. However, it is oxidized to acetaldehyde, and EtOH concentration and bactericidal effects are reduced over time¹⁷⁾. As per the results of this experiment, volatile EtOH should be an extremely effective disinfectant in cases where the object is

Table 5 Effects of NRS on colony formation of each bacteria immersed in DW with NRS

Treatment time (s)	10		30		60	
NRS (%)	0	5	0	5	0	5
<i>Escherichia coli</i>	100.0 (7.54)	99.2 (6.75)	100.0 (6.86)	101.1 (7.12)	100.0 (8.11)	98.9 (6.50)
<i>Streptococcus mutans</i>	ND	ND	100.0 (8.89)	98.67 (8.92)	100.0 (8.36)	100.3 (9.60)
<i>Staphylococcus aureus</i>	ND	ND	100.0 (7.54)	99.2 (6.75)	100.0 (6.86)	101.1 (7.12)

$n=8$ for each experimental condition. Values are mean(% of each DW treatment group) and (SD).

NRS; normal rabbit serum, ND; not done

Superscript small and capital letters denote statistically significant differences ($p<0.01$)

between the NRS concentration in same treatment time, and between the treatment time in the same NRS concentration, respectively.

Fig. 4 Photographs of the surfaces of disinfectant-immersed dental steel burs. RCC; residual chlorine concentration, (); Measured values at start point of the experiments, White lines in the micrographs represent 100 μ m.

immersed, but not wiped (Table 4). EtOH is most commonly used to disinfect objects and hands by direct application, which may lead to hand-roughening and allergy issues. Approximately 6–7% of individuals have allergic symptoms against EtOH¹⁸. Furthermore, in addition to the hazards posed by the flammability of alcohol, adverse health effects are more likely with exposure to alcohol-based hand sanitizers with aspiration of volatiles and are worse than exposure to non-alcohol-based hand sanitizers, with a higher risk especially in children under 12 years of age^{19,20}. The World Health Organization has also noted the risks/hazards of alcohol-based hand rubs²¹.

SH is widely used as a disinfectant and bleach as it is readily available at a low cost. However, SH is strongly alkaline with its unique Cl smell, and safety for humans is a big problem. Therefore, attention was focused on HA. There are various types of HA, ranging from strongly acidic to neutral pH, and it sterilizes bacteria at much lower concentration than SH. HA is capable of killing norovirus and spore bacteria, and it has less unpleasant Cl smell. However, HA is significantly degraded by UV light and heat^{10,11}; hence, it is difficult to guarantee the specifications of bottled HA (mainly the 2Mx type) sold in the market (Table 1)¹². Although the 2Mx type HA is easy to produce even at high concentrations with pH

of acidity to neutral, it is difficult to produce at home, in general facilities, and medical institutions because of the possibility of generating Cl gas during the production process (<pH 4), which can be dangerous and potentially deadly. (Fig. 1b). The 2Mx type HA is more sensitive to light than to heat, and the pH drops significantly as the RCC drops (Tables 2B a) and 3). Light shading is the first condition, when storage of the 2Mx type HA is concerned, and it can be considered usable for 1 month after production under the light shading. However, as shown in Table 1, since it is supplied in bottles, the exact date of generation cannot be determined, so care must be taken when using it. Therefore, if HA is to be used in the medical field, it should be either the EL type or CO₂ type generators; however, a proper generator, which is reasonably expensive, unlike the irresponsible cheap ones, is needed. Since HA produced by any generation method shows the same UV spectrum at the same pH²³⁾, it is basically the same HA, although there are differences in by-products and degradation over time. The 2Mx type HA was the least likely to degrade under all circumstances, followed by the CO₂ type, and the EL type was the most likely to degrade (Table 3).

The electrolysis method by a generator with bulkhead of ion exchange membrane (Fig. 1a), is difficult to produce high concentrations of HA, and RCC is usually below 50–60 ppm with strong acidic pH²³⁾. Furthermore, since the electrolysis method used electrodes and ion exchange membranes, these degrade and dissolution with use caused a decrease in performance. This requires replacement of these electrodes and ion exchange membranes, which increases the cost. Instead, it is possible to produce HA with tap water and salt, which is cheap. The EL type HA is equally affected by both temperature and light, and the RCC decreases. The RCC also decreases linearly over time in refrigerated and light-shaded products. The pH does not change much during this process. When storing EL typed HA, refrigeration and shading from light is a prerequisite, and the maximum use is within 1 week (Tables 2B b) and 3). However, the CO₂ mixing method generates HA with weak acid to neutral pH, which required a CO₂ tank, and tap water supply (Fig. 1c). Instead, high concentrations (hundreds of ppm) of HA can be easily generated by this type generator with sufficient flow rate without special maintenance. Therefore, the total running cost is lower than the EL type generator. CO₂ type HA is equally affected by both temperature and light, and the RCC decreases. The RCC decreases linearly over time, even when refrigerated and shaded from light. Unlike the 2Mx type HA, the pH does not change much or increases over time. When storing this HA, it must be refrigerated and shaded from light, and it should be used within a few weeks to 1 month (Tables 2B and 3). In any case, the primary condition for HA generation is to be generated at the site where it is used. This is supported by the results of the degradation tests in this study. The results of this storage experiment also showed that shading is more important than temperature for the storage of SH, which showed the same level of degradation over

time as 2Mx type HA. Diluted 80% EtOH is degraded more rapidly by lighting than by temperature. Alcohol concentration of unsealed 75% EtOH pads left for 21 days decreased to 47%; hence, sealing the container is considered to be an important point for storing volatile EtOH¹⁷⁾. SH and EtOH are not degraded at least within 30 days, if stored in a properly sealed shading container in a refrigerator.

The disinfectant effects of SH, HA, and EtOH on one bacteria in the oral cavity (*S. mutans*) and two bacteria associated with nosocomial infections (*S. aureus* and *E. coli*) were studied under the conditions with or without organic matter, *i.e.*, NRS. This experiment was performed in accordance with ASTM E2315-03 Time-Kill test, which already included 5% bacterial solution of protein-rich medium (1/20, 1:19) of each disinfectant solution at the bactericidal experimental stage. The disinfectant is applied after removing the organic dirt from the surface of the object. The results of this study also showed a decrease in the disinfection of each disinfectant in the presence of organic matter, NRS. Disinfecting power of HA decreased in the presence of organic matter^{2,24,25)}; therefore, the results of this study are reasonable. The results of this experiment showed insufficient disinfecting action of HA even at a concentration of 120 ppm, which is higher than the concentration at which the final joint statement of NITE, the Ministry of Economy, Trade, and Industry, and the Consumer Affairs Agency found HA to be effective at 80 ppm. The results of this study are more pragmatic, because the experimental conditions are more stringent than the usual testing. When the disinfectant effective in this experiment with a 30-s action was examined with *E. coli* for a shorter 10-s action, neither 1,000 ppm SH nor 200 ppm HA was sufficient for the disinfectant with addition of 5% NRS. These results indicate that effective disinfection requires removing dirt from the surface and then using the disinfectant, using a large amount of disinfectant to disinfect the surface while rinsing it off, or applying high concentrations of disinfectant to work for a certain amount of time (>30 s). However, it is not possible to completely remove the adhesion of organic matter in the actual clinical field. Hence, considering the human safety, it is important to use more than certain amount of freshly formed high concentration (>200 ppm) of HA and let it act for more than certain amount of time (>30 s).

As a result of the rusting test of dental cutting burs made of carbon steel, a small amount of rust was generated by DW and 80% EtOH, and HA of up to 100 ppm (Fig. 4). In contrast, 200 ppm produced a larger amount of rust, and 1,000 ppm SH induced a larger amount of rust. These agents should not be used especially for carbon steel, which is not a stainless steel that can suppress the rusting process as much as possible by the nonconductive film. EtOH does not cause metal corrosion frequently in a short period, whereas HA and SH start to corrode metal from the beginning²⁴⁾. Therefore, EtOH and HA should be applied to metal instruments only after knowing their materials.

Based on the results of this study, it was considered that presently HA with a certain level of concentration is suitable for use as an alternative disinfectant to DA. The results of this study indicated the following:

1. For flushing with running water: effective RCC of 100 ppm or higher with pH of 6.5 or lower
2. For wiping: effective RCC of 200 ppm or higher with pH of 6.5 or lower

Therefore, the CO₂ type HA generator is the most suitable for clinical environment, because it easily produces highly concentrated fresh HA safely at on-site. However, there are some disadvantages of the CO₂ type HA generator, such as it is directly connected to the tap water supply, which makes installation cumbersome and requires installation work, and the need to connect a CO₂ gas tank with periodic pressure checks.

HA is an extremely good disinfectant that is safe, economical, and effective, and can be an adequate substitute for DA. However, the use of HA with sufficient efficacy with minimal degradation requires compliance with proper storage and usage. There is an anion exchange method for generating HA, which is less susceptible to degradation and can handle high concentrations; however, it is not possible to generate it on-site²⁶⁾. Therefore, we are currently analyzing the newly developed light-resistant and rust-free HA²⁶⁾, which can be produced on demand easily under any circumstances at on-site without any generators, especially for medical, dental, and welfare applications.

CONCLUSIONS

The results of this study show that the disinfection effects of high concentration of HA were comparable to that of DA. HA is considered more suitable for normal use in terms of practicality, safety, and user comfort than DA.

The basic requirement for ideal use of HA is that it should not be stored and should be used immediately after generation, and the remaining solution should be discarded. The basic and best way is to use HA generated on demand at on-site. Therefore, HA generated by a method that can be generated on demand at on-site without using a generator, or HA generator to produce effective highly concentrated HA (at least RCC of 200 ppm) at on-site is needed, for medical and dental clinical environment. Alternatively, as a compromise, another way would be to use HA generated by a less degrading generation method with storage under cool and dark place.

HA is considered as an excellent, suitable, safe, and easy-to-use disinfectant for normal use in medical institutions.

ACKNOWLEDGMENTS

The authors are grateful to the Department of Dental Materials Science, Nippon Dental University at Niigata for partially covering the costs of this study. The authors are also grateful to Mr. Yusuke TABEI (Hypochlorous

acid Chemical Industrial Association) for useful advice and up-to-date, detailed information about disinfectants, including HA.

CONFLICT OF INTEREST

HA generator (KHM-1), and filter for tap water were provided by Renafine Inc..

REFERENCES

- 1) Kissler SM, Tedijanto C, Goldstein E, Grad YH, Lipsitch M. Projecting the transmission dynamics of SARS-CoV-2 through the postpandemic period. *Science* 2020; 368: 860-868.
- 2) National Institute of Technology and Evaluation (NITE), 2020, "Evaluation of the efficacy of alternative disinfection methods against novel coronavirus (Final Report)", available at: <https://www.nite.go.jp/data/000111315.pdf> (accessed X February 2021).
- 3) US Environmental Protection Agency, 2020, "List N for coronavirus (COVID-19), Disinfectants for use against SARS-CoV-2", available at: <https://www.epa.gov/pesticide-registration/list-n-disinfectants-use-against-sars-cov-2>. (accessed 12th May 2021).
- 4) Center for Disease Control and Prevention, 2018, "The National Institute for Occupational Safety and Health (NIOSH): High-level of disinfectants", available at: <https://www.cdc.gov/niosh/topics/healthcarehsp/disinfect.html>. (accessed 12th May 2021).
- 5) Stroman DW, Mintun K, Epstein AB, Brimer CM, Patel CR, Branch JD, *et al.* Reduction in bacterial load using hypochlorous acid hygiene solution on ocular skin. *Clin Ophthalmol* 2017; 11: 707-714.
- 6) Block MS, Rowan BG. Hypochlorous acid: a review. *J Oral Maxillofac Surg* 2020; 78: 1461-1466.
- 7) Albrich JM, McCarthy CA, Hurst J. Biological reactivity of hypochlorous acid implications for microbicidal mechanisms of leukocyte myeloperoxidase. *Proc Natl Acad Sci USA* 1981; 78: 210-214.
- 8) Dukan S, Touati D. Hypochlorous acid stress in *Escherichia coli*: resistance, DNA damage, and comparison with hydrogen peroxide stress. *J Bacteriol* 1996; 178: 6145-6150.
- 9) McKenna SM, Davies KJA. The inhibition of bacterial growth by hypochlorous acid. Possible role in the bactericidal activity of phagocyte. *Biochem J* 1988; 254: 685-692.
- 10) Len S-V, Hung Y-C, Erickson M, Kim C. Ultraviolet spectrophotometric characterization and bactericidal properties of electrolyzed oxidizing water as influenced by amperage and pH. *J Food Prot* 2000; 63: 1534-1537.
- 11) Feng Y, Smith DW, Bolton JR. Photolysis of aqueous free chlorine species (HOCl and OCl⁻) with 254 nm ultraviolet light. *J Environ Eng Sci* 2007; 6: 277-284.
- 12) Tomita T, Kikuchi K. *In-vitro* bactericidal and virus-inactivating effects of various hand-wash and gargle products. *Kansenshogaku Zasshi* 2018; 92: 670-677.
- 13) Japanese Ministry of Health, Labour and Welfare, 2009, "Documents on the designation of hypochlorite water as a food additive", available at: <https://www.mhlw.go.jp/shingi/2009/08/dl/s0819-8m.pdf> (accessed 12th May 2021).
- 14) Japanese Ministry of Health, Labour and Welfare, 2009, "Guidelines for infectious disease control in nursery schools", available at: https://www.mhlw.go.jp/bunya/kodomo/pdf/hoiku02_0001.pdf (accessed 12th May 2021).
- 15) Lu M, 2020, "The front line: visualizing the occupations with the highest COVID-19 risk", available at: <https://www.visualcapitalist.com/the-front-line-visualizing-the-occupations-with-the-highest-covid-19-risk/> (accessed 12th May 2021).

- 16) SoftBank Group Corp., 2020, “Preliminary results of antibody tests, etc”. available at <https://group.softbank/system/files/pdf/antibodytest.pdf> (accessed 12th May 2021).
- 17) Sato S, Kudo S, Kanamaru S, Saitoh Y, Yamauchi H, Tsushima R. Studies on temporal change of bactericidal activity of alcohol pad after preparation. *Jpn J Infect Prev Contr* 1991; 6: 35-39.
- 18) Stotts J, Ely WJ. Induction of human skin sensitization to ethanol. *J Invest Dermatol* 1977; 69: 219-222.
- 19) Loren Bonner L. CDC report calls attention to hand sanitizer risk in children. *Pharmacy Today* 2017; 23: 34.
- 20) Santos C, Kieszak S, Wang A, Law R, Schier J, Wolkin A. Reported adverse health effects in children from ingestion of alcohol-based hand sanitizers —United States, 2011–2014. *MMWR* 2017; 66: 223-226
- 21) WHO guidelines on hand hygiene in health care (advanced draft—World alliance for patient safety), global patient safety challenge 2005–2006: “Clean Care is Safer Care ” available at https://www.who.int/gpsc/5may/tools/who_guidelines-handhygiene_summary.pdf (accessed 12th May 2021).
- 22) Nakagawara S, Goto T, Nara M, Ozawa Y, Hotta K, Arata Y. Spectroscopic characterization and the pH dependence of bactericidal activity of the aqueous chlorine solution. *Anal Sci* 1998; 14: 691-698.
- 23) Aoki H, Miyasaka T, Ishida Y, Aoyagi Y, Miura D. Effects of hypochlorous acid electrolyzed water on dental alloys. *J Jpn Dent Mater* 2018, 37:171-178.
- 24) Takehara A, Urano H, Fukuzaki S. Cleaning of alumina fouled with bovine serum albumin by the combined use of gaseous ozone and alkaline electrolyzed water. *Biocontrol Sci* 2001; 6: 103-106.
- 25) LeChevallier MW, Cawthon CD, Lee RG. Inactivation of biofilm bacteria. *Appl Environ Microbiol* 1988; 54: 2492-2499.
- 26) Kameda T, Tabei Y, Igawa J. Instruction manual for hypochlorous acid: a review for appropriate selection and usage for extremely effective infection prevention in a clinical environment. *J Jpn Dent Assoc* 2021; 74: 33-42.