Clinical Evaluation of Chlorine Dioxide for Disinfection of Dental Instruments

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This study aimed to clinically evaluate the disinfection efficacy of chlorine dioxide (ClO_2) for used dental instruments. An imprint culture technique demonstrated that ultrasonic cleaning of intraorally applied dental mirrors in 0.02% ClO_2 for 10 minutes resulted in compete removal of microorganisms for 10 subjects. Hepatitis C virus (HCV) RNA was detected by real-time polymerase chain reaction on periodontal curettes after subgingival scaling in four HCV-infected patients and was completely removed by the same treatment procedure. Therefore, the combination of ultrasonic cleaning with ClO_2 may provide an alternative to toxic disinfectants, such as glutaraldehyde and sodium hypochlorite, for disinfecting dental instruments. *Int J Prosthodont 2013;26:541–544. doi: 10.11607/ijp.3465*

Preventing the transmission of infectious microorganisms during dental procedures is important for infection control.[1] Various disinfectants are commercially available, and specific recommendations concerning their use are mainly based on in vitro studies of the effectiveness of disinfection procedures against bacteria and fungi (Table 1). Chlorine dioxide (ClO_2) is considered a reliable oral disinfectant because of its strong sterilizing and tissue-dissolving properties.[2] ClO_2 does not form toxic chemical derivatives as do chlorine and hypochlorite and, therefore, is safer for the human body than conventional disinfectants such as glutaraldehyde (GA) and sodium hypochlorite (NaOCl). However, disinfection of dental instruments by ClO_2 has been primarily determined in vitro, and evidence has not been obtained in clinical settings. Thus, the aim of this study was to clinically examine the efficacy of ClO_2 in disinfecting dental instruments after use.

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Materials and Methods

This study was conducted in accordance with a protocol approved by the Ethical Committee of the Osaka University Graduate School of Dentistry (H23-E5). First, the effectiveness of a minimal fungicidal concentration (MFC) of ClO_2 against *Candida albicans* planktonic and biofilm cells (10^7 cells/mL) was evaluated using the XTT (2,3-bis-[2- methoxy-4- nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide) assay.[3] The MFC values for ClO_2 in planktonic (0.0078%) and biofilm (0.016%) modes were less than 0.02% and, thus, noticeably lower than the values for NaOCl (0.023% and 0.19%, respectively).

The efficacy of 0.02% ClO_2 was compared with that of commercially available disinfectants (2% GA and 1% NaOCl) in removing microorganisms from dental mirrors that had been placed in the mouths of patients (touching the surface of the buccal mucosa) for 1 minute (Fig 1a). The subjects included 10 adults (4 women and 6 men, mean age: 26.9 years, range: 24 to 35 years) randomly selected from patients at the Department of Fixed Prosthodontics, Osaka University Dental Hospital, Osaka, Japan. Eight autoclaved dental mirrors were used for each patient, and seven mirrors underwent one of the disinfection treatments shown in Table 2 (with or without ultrasonic cleaning, Fig 1b); the remaining mirror was left untreated as a control. The presence of oral microorganisms on the dental mirrors was evaluated using an imprint culture technique (Fig 1c) as described in a previous study.[4]

We further assessed whether ultrasonic cleaning in 0.02% ClO_2 removes viral (eg, hepatitis C [HCV]) contamination on dental instruments. Subgingival scaling

Table 1 Publications on Disinfection Treatments in the Field of Dentistry

Publication	Disinfection method	Study method	Samples	Detected microorganisms	Results
Angelillo et al[6]	1% peroxygen, 2% AG	In vitro	Dental instruments	Bacteria, fungi	2% AG is recommended for chemical sterilization or high-level disinfection
Furukawa et al[7]	Chlorine dioxide	In vitro	Soft denture liners attached to acrylic resin bases	Bacteria, fungi	Chlorine dioxide did not achieve the minimal standard of disinfection for soft denture liners
Bettner et al[8]	Enzymatic detergents, ultrasonic bath	In vitro	*Streptococcus mutans* suspensions	*S mutans*	Ultrasonic cleaning effectively reduced bacterial contamination
Leontiou et al[9]	2.4% AG, asepsys, chlorine dioxide, propyleneglycol, oxone, ultrasonic cleaning	In vitro	Dental diamond burs contaminated with serum from HBV-positive patients	HBV	Disinfectants should be used with ultrasonication to inactivate HBV
Ramadan[10]	Scrubbed with a toothbrush	In vitro	PTFE-coated orthodontic archwires immersed in HCV-infected blood	HCV	PTFE-coating inhibited HCV adhesion when thoroughly scrubbed
Wichelhaus et al[11]	70% isopropanol, ethanol, and propanol, glucoprotamine, ultrasound bath, steam disinfection	In vitro, in vivo	Weingart pliers and distal-end cutters	Bacteria (in vitro/in vivo), fungi, viruses (in vitro)	Ultrasound bath and thermal disinfection is recommended for disinfection
Perakaki et al[12]	Ultrasonic bath, washer disinfector	In vitro	Endodontic files used in extracted teeth	Residual debris	Less residual debris after cleaning in an ultrasonic bath than after cleaning in a washer disinfector
Egusa et al[4]	2% AG, 1% SH, 0.25% BC, 1 ppm ozonated water, hygojet/MD520 system	In vivo	Dental impressions	Bacteria and fungi	The combined use of BC with general disinfectants is recommended

AG = alkaline glutaraldehyde; SH = sodium hypochlorite; BC = benzalkonium chloride; HBV = hepatitis B virus; HCV = hepatitis C virus; PTFE = polytetrafluoroethylene.

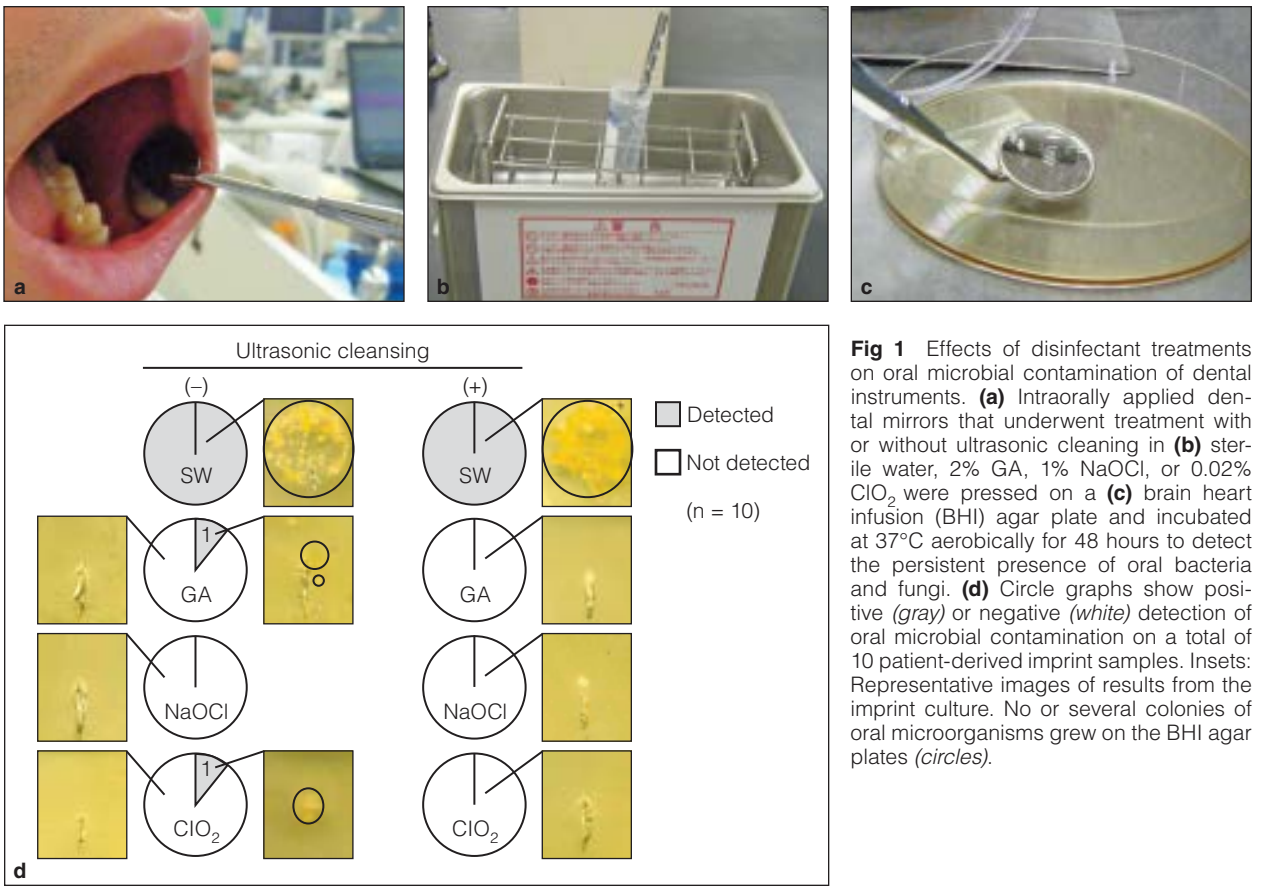
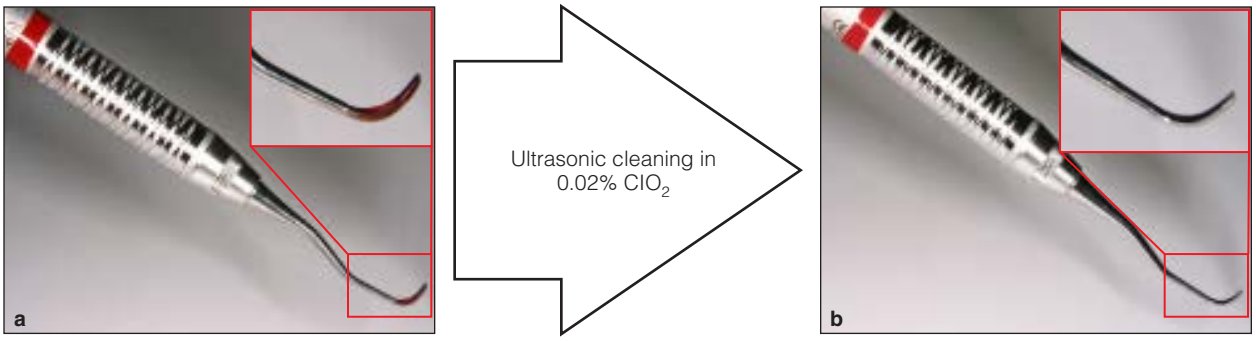


Fig 1 Effects of disinfectant treatments on oral microbial contamination of dental instruments. **(a)** Intraorally applied dental mirrors that underwent treatment with or without ultrasonic cleaning in **(b)** sterile water, 2% GA, 1% NaOCl, or 0.02% ClO_2 were pressed on a **(c)** brain heart infusion (BHI) agar plate and incubated at 37°C aerobically for 48 hours to detect the persistent presence of oral bacteria and fungi. **(d)** Circle graphs show positive *(gray)* or negative *(white)* detection of oral microbial contamination on a total of 10 patient-derived imprint samples. Insets: Representative images of results from the imprint culture. No or several colonies of oral microorganisms grew on the BHI agar plates *(circles)*.

Table 2 Disinfection Treatments of Dental Instruments

Disinfectant	Manufacturer	Procedure	Ultrasonic cleaning
Sterile water (UT-106H)	Sharp	Immersion for 10 min	+ or –
0.02% chlorine dioxide	Daiso	Immersion for 10 min	+ or –
2% glutaraldehyde (Sterihyde L)	Maruishi Pharmaceutical	Immersion for 10 min	+ or –
1% sodium hypochlorite	Yoshida Pharmaceutical	Immersion for 10 min	+ or –



Subject	No treatment (log IU/mL)	Ultrasonic cleaning in 0.02% ClO_2
A	3.5	Not detected
B	4.6	Not detected
C	2.0	Not detected
D	2.5	Not detected
Detection range: 1.08–8.00 log IU/mL		

c

Fig 2 Effects of ultrasonic cleaning with ClO_2 on the removal of HCV from HCV-infected patient-derived dental instruments. After subgingival scaling on molars, **(a)** blood and saliva adhered to the surface of a periodontal curette and **(b)** were visually removed by ultrasonic cleaning in 0.02% ClO_2. **(c)** Real-time PCR detected HCV RNA in instruments that had been used in four HCV-infected patients (2.0–4.6 log IU/mL). In contrast, HCV RNA was not detected in the instruments after ultrasonic cleaning in 0.02% ClO_2.

was performed on the molars of four HCV-positive patients (1 woman and 3 men, mean age: 70.8 years, range: 60 to 77 years) using two autoclaved periodontal curettes. One curette then underwent ultrasonic cleaning in 0.02% ClO_2 for 10 minutes, and the other was left untreated. The presence of HCV was evaluated using real-time polymerase chain reaction (PCR).

Results and Discussion

Disinfection using 2% GA or 0.02% ClO_2 resulted in the complete removal of visible colonies for nine samples, whereas a small portion of a colony was detected in one sample for each treatment (Fig 1d). Disinfection using 1% NaOCl resulted in complete removal of visible colonies from all samples. Notably, ultrasonic cleaning in sterile water did not effectively reduce colony growth relative to untreated control samples. In contrast, treatment with all disinfectants used in the study together with ultrasonic cleaning resulted in the complete removal of visible colonies in all samples. Ultrasonic cleaning is an appropriate cleaning procedure for dental instruments as opposed to manual scrubbing, which may cause the spread of microbial contamination and result in skin-puncture injuries.[5] However, the present results indicate that treatment of dental instruments by ultrasonic cleaning without the use of a disinfectant is inadequate. Therefore, application of 0.02% ClO_2 during the ultrasonic cleaning of dental instruments is recommended to achieve complete removal of oral bacteria and fungi without using toxic disinfectants, such as GA and NaOCl.

In the HCV-positive patients, blood and saliva were attached to the instruments after manual scaling (Fig 2a). After ultrasonic cleaning in 0.02% ClO_2,

no attached blood was visible (Fig 2b). Notably, HCV RNA was detected in all untreated patient samples (Fig 2c), which indicates a potential risk of transmission of the virus to dental staff and other patients. Viral contamination was completely removed by the ultrasonic cleaning in 0.02% ClO_2 in all samples. However, it must be noted that the study population for HCV-positive patients was small; thus, the results reported here may be specific to this study sample.

It is unclear whether the disinfectant procedure has the same effect on the hepatitis B or human immunodeficiency virus, both requiring clinicians to pay attention to the risks of cross-infection. Additional research is required to establish a robust disinfection procedure in light of new scientific evidence under clinical conditions.

Conclusion

Taken together, these results demonstrate that contaminant microorganisms and viral particles were present on instruments even after ultrasonic cleaning in sterile water. Therefore, used dental instruments must be assumed to have the potential to transmit infectious agents to dental personnel. From the standpoint of microbiologic effectiveness and safety, ultrasonic cleaning of dental instruments in 0.02% ClO_2 for 10 minutes prior to autoclaving would be recommended for clinical use as a promising procedure for reliable infection control.

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References

1. Egusa H, Watamoto T, Abe K, et al. An analysis of the persistent presence of opportunistic pathogens on patient-derived dental impressions and gypsum casts. Int J Prosthodont 2008;21:62–68.
2. Lundstrom JR, Williamson AE, Villhauer AL, Dawson DV, Drake DR. Bactericidal activity of stabilized chlorine dioxide as an endodontic irrigant in a polymicrobial biofilm tooth model system. J Endod 2010;36:1874–1878.
3. Watamoto T, Samaranayake LP, Jayatilake JA, Egusa H, Yatani H, Seneviratne CJ. Effect of filamentation and mode of growth on antifungal susceptibility of *Candida albicans*. Int J Antimicrob Agents 2009;34:333–339.
4. Egusa H, Watamoto T, Matsumoto T, et al. Clinical evaluation of the efficacy of removing microorganisms to disinfect patient-derived dental impressions. Int J Prosthodont 2008;21:531–538.
5. Popovic J, Gasic J, Zivkovic S, Petrovic A, Radicevic G. Evaluation of biological debris on endodontic instruments after cleaning and sterilization procedures. Int Endod J 2010; 43:336–341.
6. Angelillo IF, Bianco A, Nobile CG, Pavia M. Evaluation of the efficacy of glutaraldehyde and peroxygen for disinfection of dental instruments. Lett Appl Microbiol 1998;27:292–296.
7. Furukawa KK, Niagro FD, Runyan DA, Cameron SM. Effectiveness of chlorine dioxide in disinfection on two soft denture liners. J Prosthet Dent 1998;80:723–729.
8. Bettner MD, Beiswanger MA, Miller CH, Palenik CJ. Effect of ultrasonic cleaning on microorganisms. Am J Dent 1998;11: 185–188.
9. Leontiou AP, Coogan MM, Aspinall S. Disinfection of dental diamond burs contaminated with hepatitis B virus. J Prosthet Dent 1999;82:332–335.
10. Ramadan AA. Removing hepatitis C virus from polytetrafluoroethylene-coated orthodontic archwires and other dental instruments. East Mediterr Health J 2003;9:274–278.
11. Wichelhaus A, Bader F, Sander FG, Krieger D, Mertens T. Effective disinfection of orthodontic pliers. J Orofac Orthop 2006; 67:316–336.
12. Perakaki K, Mellor AC, Qualtrough AJ. Comparison of an ultrasonic cleaner and a washer disinfector in the cleaning of endodontic files. J Hosp Infect 2007;67:355–359.

Literature Abstract

Do angulated implants increase the amount of bone loss around implants in the anterior maxilla?

The purpose of this prospective study was to investigate the relationship between angulated implants and the amount of bone loss around implants in the anterior maxilla. The subjects had a missing tooth in the anterior maxilla and a bone deficiency that required restoration with an angulated dental implant. Radiographic examination was performed in each patient immediately after loading and repeated a minimum of 36 months after loading. Fifty-eight subjects (38 men, 20 women; mean age = 26.8 years) who received delayed-loading angulated implants were evaluated. The results showed that the mean implant angulation was 15.2 degrees and the mean bone resorption was 0.87 mm. There was significant correlation between implant follow-up time and bone loss. No correlation was found between the implant angulation and bone loss. An assessment of predictive factors showed a relationship between the implant type and bone loss. The angulated implants did not increase bone resorption on the mesial and distal surfaces of the implants. The authors concluded that the implant angulation was not correlated with an increased risk for bone loss, and angulated implants may be a satisfactory option to vertical implants to avoid grafting procedures. The type of implant may be a significant factor that affects bone resorption. Follow-up time was, however, the strongest predictive factor.

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