Environmental Monitoring and Bactericidal Efficacy of Chlorine Dioxide Gas in a Dental Office

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We monitored the quantity of airborne microorganisms at 11 points (points A to K) in a dental office on a routine day of use, and tested the bactericidal efficacy of chlorine dioxide (ClO2) gas in the dental operatory after consulting hours. Fallen airborne microorganisms were collected under air-conditioning (AC) in the dental office, and under four conditions in the operatory. Specimens of the microbes were cultivated on nutrient and Sabouraud agar media (NAM and SAM). Many colonies were observed at the entrance hall and on the cabinet in a disinfection room in the NAM and SAM tests, respectively, while no colony was observed at the foot position of the operating table and treatment bed, and above the head position of the operating room in the NAM and SAM tests, respectively. In the bactericidal efficacy test using ClO2 gas, the dental operatory could be kept clean by the use of 4 mg/L-CIO2 gas in addition to the use of an AC with a plasma filter and the HEPA filter.

Key words: Chlorine dioxide gas/Bactericidal effect/HEPA filter/Air-borne-microbe.

In dental offices where implant procedures are performed, what is of paramount importance is to avoid horizontal or cross transmission of infectious agents. Taking antimicrobial prophylactic measures is the first step toward the prevention of infections in clinical practice (Komai, 1995; Szymanska, 2004; Klevens, 2008). Today, it is common that patients who undergo implant surgery are ambulatory patients. The utmost care must be taken because there is a risk of patients being exposed to infectious germs (Garg et al., 1994). Therefore, dental offices are urged to appropriately take standard precautions to break chains of transmission of pathogenic microorganisms (Council on Dental Materials and Devices and Council on Dental Therapeutics, 1978; Hamann, 2004). Infection control is indispensable not only in surgery but also in prosthetic (Furukawa et al., 1998; Lin et al., 1999; Hamann, 2004) and orthodontic therapy (Cash, 1988).

Opportunistic pathogens causing nosocomial infections are transmitted to patients by way of various kinds of media in offices or surgical procedures. As one of the prophylactic measures against nosocomial infections, chlorine dioxide (ClO2) had been used on the front line of medical care to disinfect hemodialyzers, and gastrointestinal and nasal endoscopes (Working Party Report, 1998; Coates, 2001; Street et al., 2006). On the other hand, ClO2 is used for the sterilization of waiting and laboratory practice rooms in clinics, of beds, bedclothes and mattresses in sickrooms, and of ambulance cars (Takayama et al., 1995; Aoyagi, 2002; Hernandez et al., 2008; Umeda and Okuda, 2009). In recent years, the disinfection and sterilization practices using ClO2 initiated by medical supplies manufacturers and nursing care facilities from the point of view of good environmental hygiene have attracted a great deal of

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attention (Takayama et al., 1995; Taguchi, 2000; Sagara and Kowalski, 2000; Coats, 2001; Han et al., 2003; Isomoto et al., 2006; Hernandez et al., 2008).

In the domain of dental practice, it is the general practice to use ClO₂ solutions to disinfect scaling devices, dental unit waterlines and ultrasonic units. The solutions are also used in a medicine for chronic atrophic oral candidiasis and treatment of halitosis (Yaegaki and Coil, 2000; Mohammad et al., 2004). ClO₂ is also reported to prove efficacious in controlling nosocomial infection-causing bacteria such as methicillin-resistant Staphylococcus aureus (MRSA), enterotoxigenic Escherichia coli (E. coli), O-157, (Takayama et al., 1995; Taguchi, 2000; Sagara and Kowalski, 2000; Coates, 2001; Aoyagi, 2002; Kimoto et al., 2004; Isomoto et al., 2006; Hernandez et al., 2008) spores, (Han et al., 2003) Legionella, (Szymanska, 2004) Porphyromonas gingivalis (Pg), (Kubota et al., 2005) and some viruses (Thurston-Enriquez, 2005). In addition to these beneficial properties, ClO₂ has the advantage of being available at a relatively low price. In addition, there have been no reports that ClO₂ generates carcinogenic organic re-
siduals (Sagara and Kowalski, 2000; Svecevicius et al., 2005). However, there is hardly any study available that investigates the effective use of ClO₂ in cleaning the dental surgery (Palenik et al., 2000) and implant surgery rooms, (Garg et al., 1994) and in disinfecting and sterilizing implant materials, surgical instruments and tools (Kubota et al., 2005). The objectives of the present study were to check the quantity of airborne microorganisms that fall in a dental office, and to assess the bactericidal efficacy of using devices such as an air-conditioning unit with a plasma filter, a high-efficiency particular air filter and ClO₂ gas generator in a dental operatory.

The experiment was carried out at a dental office where implant therapy is performed. Figure 1 shows the floor plan of the office and 11 monitoring points (points A to K). The office has an entrance hall used also as the waiting room, a consultation room, a dental operatory, a disinfection and sterilization room, a dental laboratory and an X-ray room. All the tests were done during consultation hours. Samples of airborne microorganisms were cultivated on nutrient agar media (NAM) and Sabouraud agar media.

**FIG. 1.** The floor plan showing the 11 monitoring points (A to K) in the dental office
AC: Air-conditioning
α and β: Bactericide-ClO₂ gas generators
ACP: Air-conditioning with a plasma filter
†: HEPA (high-efficiency particular air) filter
(SAM) in petri dishes. At all the monitoring points, three pairs of dishes, each containing one of the two types of media, were set up. The dishes were actually placed on the cabinets of the dental units or the mobile wagons in the office, a little over 90 cm above the floor (Itani et al., 2005; Tsuji et al., 2009). The dishes were let to stand for 45 minutes with the lids off. Thereafter, the dishes were covered with the lids and put into the containers for incubation: NAM for 48 hours at 37 °C and SAM for five days at 30 °C (Furuzumi, 1989). The number of colonies of microbes that multiplied on the media was counted with the naked eye.

In monitoring the environmental hygiene (Experiment 1), airborne microorganisms were collected at 11 points (points A to K) in the office as shown in Fig. 1. Collections were done with the air-conditioning (AC) in every room except the operating theater. A Yammer AC (Y4GPBM, Yammer, Osaka, Japan) using a gas heat pump was in operation in the consultation room and two units of Tokyo Gas AC (TS-B2842U, TOKYO GAS, Tokyo, Japan) were used in the dental laboratory and the waiting room. The door to the dental operatory was kept closed.

In monitoring the environmental hygiene in the dental operatory (Experiment 2), airborne microbes were collected at three points in the dental operatory after the daily dental practice had been completed. The room was equipped with an air-conditioning unit with a plasma filter (ACP) (Kirigamine®, Mitsubishi Electric, Tokyo, Japan) and a high-efficiency particular air (HEPA) filter (Idealize Medical Construction, Tokyo, Japan). The ACP had a double filter. The HEPA filter was operated under the following conditions: a wind volume of 14 m³ per minute, a wind velocity of 0.45 m per second and a cleaning capacity of 840 m³ per hour. It was installed at the ceiling above the head of the operating table so that the air would blow toward the head of HEPA filter vertically. The air outside entered by way of the air intake pipe with the ventilating fan (V-12 PSEQD4, Yammer, Osaka, Japan) fitted on the left corner of the ceiling, passed through the filter installed on the ceiling right above the foot (point C) of the operating table, and entered the HEPA filter, which sent pure and clean air circulating in the room. The air spontaneously left the operatory, where positive pressure was maintained, through the air vents on the lower part of the semi-automatic door toward the consulting room as shown in Fig. 1.

The airborne bacteria were collected under the following conditions after consulting hours:
(a) After the ACP and the HEPA filter were used simultaneously for 30 minutes;
(b) After the bactericide units were used together with the ACP and the HEPA filter for 30 minutes;
(c) After the simultaneous use of the three types of devices for 30 minutes as in condition b, only the bactericide units were switched off, the door of the dental operatory was opened and shut six times within a space of two minutes and a worker went back and forth between the consulting room and the dental operatory;
(d) After condition b was instituted and the only bactericide units were turned off, the door to the operating room was closed for three hours.

The Cl₂ gas used in this experiment was generated by using two bactericide units (SCB 001A, NISHIKAWA, Tokyo, Japan). The two gas generators were installed side by side along the midsection of the opposite side wall in the dental operatory. As shown in Fig. 1, the gas was sent out from the windows on the both sides of the bactericide units. The gas concentration for this device was 2 mL/L. 5 ml of stabilized Cl₂ solution (60,000 mg/L) were added to 500 g of gel stabilized Cl₂ (20,000 mg/L) available from the market so that 4 mg/L Cl₂ gas was prepared. The concentration of the gas generated was collected by a vapor collector (Model GV-100, Gastec, Tokyo, Japan) and its concentration was measured using a gas detector tube (No. 23M, Gastec, Tokyo, Japan). The operating theater measured 2.9 meters in width, 3.8 meters in depth and 2.3 meters in height (cubic capacity: 25.3 m³). The door connecting the operating theater with the consulting room was semi-automatic.

The number of colonies of airborne bacteria cultured on each medium in the three pairs of dishes was counted, and the average number for NAM and SAM was worked out for use as the number of colonies grown on each type of media.

Table 1 shows the distribution of the colony counts of airborne organisms grown in the two types of medium (NAM and SAM) measured at the 11 points. The NAM tests for airborne bacteria revealed 7.6 colonies at point K, which was the largest of all. On the other hand, at points B and D, no colony of airborne bacteria was recognized. Likewise, SAM tests found the largest 6.3 colonies formed at point I. No colony was formed at point C.

As shown in Table 2, the examinations at three points within the dental operatory found that when the ACP was on (condition a), the average number of colonies came to 0.3-1.7 in the NAM tests and 0-2.0 in the SAM tests. When the ACP, HEPA filter and Cl₂ gas were used at the same time, the formation of colonies was not recognized in either type of medium (condition b). However, when the use of Cl₂ gas
was suspended and the door of the dental operator was opened and shut, the NAM tests found 0-1.7 colonies and the SAM tests found 0-2.0 colonies (condition c). On the other hand, when the door was closed for three hours after stopping only the bactericide units, no colony was observed in either test (condition d).

The results of the environmental monitoring test during consultation hours clearly showed that there were places where at the same monitoring site the number of colonies on one type of media was similar to that on another type of media or quite different between the two. At the entrance hall (point K), the number of microorganisms was the largest on either NAM or SAM culture medium. It was also found that there were places free from contamination - - at point B and point D in the NAM tests and point C in the SAM tests. However, there was no place where microorganisms were undetected at all on the media (Table 1). From the results of the experiments conducted in the dental operator, the fact that microorganisms were not detected at any place could be attributable to the simultaneous use of the ACP, HEPA filter and the bactericide units (conditions b and d) (Table 2).

In the dental office where implant surgery and other surgical procedures are routinely performed, every precaution has to be exercised against wound infection, horizontal or cross infection, and iatrogenic infection as well (Takayama et al., 1995; Kronstrom et al., 2000; Sagara and Kowalski, 2000; Taguchi, 2000; Coates, 2001; Aoyagi, 2002; Han et al., 2003; Kimoto et al., 2004; Szymanska, 2004; Kubota et al., 2005; Isomoto et al., 2006; Hernandez et al., 2008). We used the method of measuring airborne bacteria as a means to investigate the cleanliness of the environment in a dental office (Furuizumi, 1989, Ichiman et al., 2000; Itani et al., 2005; Suzuki, 2008; Tsuji et al., 2009). In recent years, new firms have been established to regularly clean operating theaters and intensive care units in hospitals and clinics on contract. They strategically use a method similar to the one that we employed to check the state of ambient hygiene by monitoring the quantity of airborne microorganisms.

In our experiment 1, plenty of airborne microbes were detected at points A, E, H, J, and I, respectively (Table 1). There are many possible reasons for this. The microbes could take the opportunity of invading the office when patients visit. Some microbes might enter the office together with fine particles on the patients' clothes. The movement of patients, the activity of the dental office personnel, the turbulence of the ambient airflow current by air-conditioning, and the
scattering in air of skin organisms could be also contributing factors (Hambraeus, 1988). Furthermore, the grinding of teeth, and the removal of dental calcareous deposits and biofilm by using ultrasonic or air-scalers might have caused fine particles and bacteria to scatter around environmental surfaces within the dental operatory or the consulting room (Alms, 1976; Palenik et al., 2000).

It is also conceivable that our findings at points E and H might have been affected by the inflow of the above-mentioned minute and suspended pollutants in the air from ACs (Table 1). There is a report that infection-causing microorganisms in dental clinics were detected in dental materials that had been transported to a remote place (Powell, 1990). In our experiment, it was found that microbes, which adhered to used prosthetic appliances and impression materials, stayed on the border in the boundaries between the consulting room and the dental laboratory (Furukawa et al., 1998; Verran, 2004). From these results, it can be said that it is necessary to keep the floor clean in the dental office and to contrive a means to make the air current flow in a fixed direction.

In the culture tests using SAM agar medium, a plethora of microorganisms were detected at points H, I and J (Table 1). The conceivable factors behind this are that the steam from the autoclave sterilizer, the damp and dust from the dental laboratory, and in addition, inadequate ventilation, gave rise to the multiplication of microbes. In the future, it would be necessary to give more thought to the ventilation system and the management of humidity in the disinfection and sterilization room, laboratory and the entrance hall.

On the other hand, both of NAM and SAM culture tests found only a few microbes at points B and C in the operating theater and at points D and G in the consulting room (Tables 1 and 2). This is probably because the operating theater was used less frequently than the other rooms and its door was usually kept closed (Cash, 1988; Hambraeus, 1988). Generally, the number of fallen airborne microbes is influenced by the air current and wind velocity (Suzuki, 2008). In other words, the consulting room was well ventilated with the air flowing smoothly at the two monitoring points.

The monitoring of the environmental hygiene in the dental operatory (experiment 2) came up with the following findings. As a result of the simultaneous use of the ACP and the HEPA filter for 30 minutes (condition a) to improve the hygiene in the operating theater, the number of microbes decreased at points B and C (Table 2). The air in operating theaters was once reported to be contaminated by plenty of Staphylococci and Micrococci with negative coagulase (Hambraeus, 1988). As a result, it has become standard practice for hospitals to install an ultra-clean unit in the operating room. Japanese researchers investigating the colony numbers of airborne microorganisms in eight operating rooms reported that the mean number of colonies was within 1 to 6 (Furuhashi et al., 1972; Itani et al. et al., 2005). In the present study, in both NAM and SAM tests, the number of colonies was two or less, which was smaller than the figure in the above report. The colony numbers of fallen airborne microorganisms in the dental office was similar to that in the operation theatre in a medical office, and was remarkably smaller than that (under 30) of the laboratory and practical rooms in a clinic and school (Ichiman et al., 2000; Suzuki, 2008; Tsuji et al., 2009). Some of the reasons could be high temperature, small size, and rich nutritional conditions of the culture media (Shintani, 2006; Suzuki, 2008). It is also conceivable that there is a difference between the methods of collecting the microorganisms in terms of passive and active microbial samplings. In this study, we used the former method, because it is low in cost and simple to perform, but will need to use the latter system in the future.

The decreasing tendency shown in the results of tests in the dental operatory was considered to be due to the effect of the ACP and the HEPA filter which blew ultra fresh air over the area centering on the head of the operating table (point B). However, near point A, the air in the dental operatory appeared to have become stagnant as the air from the HEPA filter appeared to have caused the eddy convection to occur near the entrance. On the other hand, at point C, the number of colonies was small and almost unchanged whether the air cleaner was on or off, probably because there was a port, which let in fresh air, and a filter above the end of the operating table (Fig. 1 and Table 2).

Against the ambient air contamination caused by patients and staff, the bacteria peeled away from the skin, and the scattering of microbes in the air when patients put on or take off their clothes, the dry-type filter system like HEPA filter is effective as a means of controlling infections (Hambraeus, 1988; Safdar et al., 2005). The HEPA filter is capable of removing 99.97% of airborne particles with a diameter of 0.3 \( \mu \text{m} \) or more. Therefore, this type of device is indispensable for cleaning the operating theater used for various types of transplantation, particularly when implant surgery is performed. On top of this, it may be necessary to use an effective air-conditioning system.
and sterilized uniforms because pathogenic bacteria from teeth and periodontal tissue often cause postoperative infections (Garg et al., 1994).

The present study confirmed that the growth of microorganisms was inhibited when 4 mg/L-CIO₂ gas was used together with the ACP and the HEPA filter in the operating room. In other words, CIO₂ gas was proven to be very effective in disinfection and sterilization.

From the above-mentioned reports on the control of MRSA, E. coli, spores, and Pg (Aoyagi, 2002; Takayama et al., 1995; Sagara and Kowalski, 2000 Taguchi, 2000; Han et al., 2003; Kimoto et al., 2004; Kubota et al., 2005) and the results of our study, we think that the disinfection and sterilization of the operating theater by CIO₂ gas (4 mg/L) could be an effective strategy for promoting the cause of environmental hygiene. CIO₂ gas is considered to be a promising low-temperature antimicrobial system to replace ethylene oxide gas (EOG), which is carcino- and mutagenic (Taguchi, 2000; Sagara and Kowalski, 2000).

When the door to the dental operatory was opened and shut after the simultaneous use of three devices (condition c), the growth of once inhibited microbes was again observed at points A and B (Table 2). Conceivably, this is because the air contaminated by airborne microbes from patients' mouths, skin and clothing entered the operating room from the adjoining consulting room without being blocked by the ACP and HEPA filter (Hambraeus, 1988). The results of this study were in support of the recommendation that the operating room should be off limits to persons except those directly concerned (Ayliffe, 1991; Whitacre, 1991).

Even after the elapse of three hours following the suspension of the bactericide units after the disinfection and sterilization of the room by CIO₂ gas (condition d), it was found that the operating theater remained clean. As this finding suggests, it is very important to isolate the operating theater from the other rooms, shut the door after the exposure to CIO₂ gas, and to maintain the positive pressure within the operating theater (Komaï, 1995; Cash, 1988; Hambraeus, 1988).

When the concentration of CIO₂ gas used in this study became more than 4 mg/L, our eyes became sore and an irritating smell assailed the nasal mucosa. Accordingly, in order to inactivate infectious agents in the operation theater, we think that the stress on the living body should be kept to a minimum and that the use of low concentration CIO₂ gas is recommended.

In conclusion, to preserve the environmental hygiene at a satisfactory level in dental offices, we need to keep a watchful eye for possible air contamination in crowded places such as the entrance hall and the borders between the consulting room and dental laboratory, the scattering of pathogenic microorganisms from the mouth, the humidity level within the disinfection and sterilization room, the cutting off of the dust pollution and the supply of vapor in laboratory. The unpopulated operating room after consulting hours can be kept clean by the use of 4 mg/L of CIO₂ gas together with the ACP and the HEPA filter. In addition, measures under implant surgery should be taken so that no one except those persons concerned is allowed to enter the dental operatory. Further in vivo studies, however, should consider the decrease in the residual concentration of CIO₂ gas in the operation theatre.

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