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Technical report

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Effect of Denture Cleaner using Ozone against Methicillin-resistant *Staphylococcus aureus* and *E. coli* T1 Phage

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We examined the bactericidal and virucidal effectiveness of a denture cleaner that uses ozone (ozone concentration, 10 ppm) against methicillin-resistant *Staphylococcus aureus* (MRSA) and T1 phage, respectively. In the bactericidal activity test, with the ozone supply turned on, the number of bacteria was 3.1×10^3 CFU/mL at the beginning of the experiment, fell to 1.0×10^0 CFU/mL 10 min later, and was 1.0×10^0 CFU/mL or less afterwards. In contrast, when the ozone supply was cut off (air bubble only), the number of bacteria was 3.4×10^3 CFU/mL at the beginning of the experiment, and had fallen to 3.0×10^3 CFU/mL 60 min later (no statistically significant difference). In the virucidal activity test, the number of phages was 1.2×10^6 PFU/mL before ozone treatment, fell to about 1/10 of that number 10 min later, and was 6.1×10^0 PFU/mL 40 min later.

These results indicate that the use of ozone in this denture cleaner is effective against MRSA and viruses.

Key words : Ozone, MRSA, *E Coli* T1 Phage

INTRODUCTION

Currently, the cleaning methods used by denture cleansers are mainly to disinfect or sterilize removable dentures. However, cases of accidental drinking of denture cleanser by small children and elderly people or of other misuse have been reported[1,2]. Therefore, we have been searching for a safer denture cleaning method and as a result, have developed a denture cleaner employing ozone[3,4]. If accidental drinking of the low concentration ozone water occurs, the ozone will be dissipated by oxidation of the saliva or combustion in the oral cavity and only water will remain.

Recently, methicillin-resistant *Staphylococcus aureus* (MRSA) and viral infections have become problematic in the field of dentistry. MRSA causes hospital infections, and has also been detected at extremely high levels in decubitus ulcers and the throats of bed-ridden elderly. The elderly are the age group most likely to use dentures, which they often clean after meals at the bedside or in the bathroom. It is thought that, when the dentures of hospitalized elderly patients are cleaned, there is a possibility of hospital staff or other elderly patients contracting infection from the

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dentures or the denture brush. The immune system of the elderly is relatively weak, making infection easier and cure more difficult.

Also, patients visiting their dentist sometimes have viral infections such as hepatitis or AIDS, and these patients may need new dentures or may need to have their existing dentures adjusted. Even though infection-prevention measures are taken as far as possible, there is a limit to what measures can be taken with outpatients. Therefore, from the standpoint of reducing the chance of infecting doctors, staff or other patients, it is desirable to disinfect and sterilize patients' dentures before treatment.

With this in mind, we studied the effects of a denture cleaner we developed on MRSA and viruses. We used the *E. coli* T1 phage to test antiviral effects because of its physiochemical resemblance to enterovirosuses.

MATERIAL AND METHODS

Denture cleaner and ozone measurement

The ozone denture cleaner (Fig. 1) used in this experiment consisted of an ozonizer (ozone concentration, 10 ppm) and a tank in which dentures were placed. The details of this apparatus are described in previous reports[3,4]. The ozone concentration was measured with an ozone measurement gauge (OZM-G21-ZW, Okitoronics Co., LTD., Tokyo, Japan) and a liquid ozone concentration meter (OZ-20, Toa Dengyo Co., LTD, Tokyo, Japan) was used to determine if the concentration of ozone dissolved in water was below the range of possible measurement.

Tests of bactericidal and virucidal effectiveness

1. Test of bactericidal effectiveness

MRSA ID116778 (obtained from Tokyo Univ.) was used in this experiment. MRSA

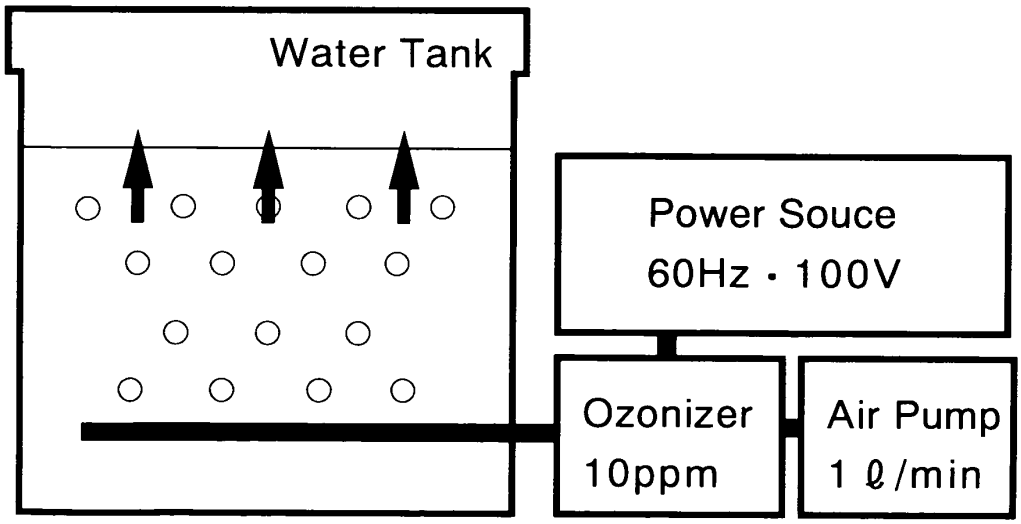

Fig. 1 Illustration of denture cleaner using ozone.

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was streaked on plates with heart fusion agar culture medium using a platinum loop, and cultured at 37℃ for 24 hr.

The bacteria grown on the plate medium were scraped up with a loop and suspended in 10 mL sterile water. The turbidity of this suspension was adjusted to 0.4 at 420 nm using a spectrophotometer (Shimazu Co., LTD. Kyoto, Japan). An aliquot (0.2 mL) of this suspension was added to 2 L sterile water and stirred well. This was the bacterial solution used in the bactericidal tests.

For each test, 700 mL of the bacterial solution was poured into the cleaner tank. A 0.1-mL sample (before operation of the machine) was then withdrawn from the tank, and the cleaner was switcheden. Thereafter, over a period of 60 min, a 0.1-mL sample of the bacterial solution was withdrawn every 10 min. Each sample was diluted 10 fold and cultured at 37℃ for 24 hr.

After culturing, the number of colonies on each plate was counted, and this was used to calculate the number of MRSA bacteria in 1 mL of solution. As a control experiment, this test was repeated with the supply of ozone cut off (air bubble only). During the experiments, the room temperature was 28℃ and humidity was 42%.

2. Test of virucidal effectiveness

E. coli was inoculated into a nutrient bouillon and cultured at 36℃ for 24 hr.

E. coli (IFO 13168) and *E. coli* T1 Phage (IFO 20001) were inoculated into a nutrient bouillon and cultured at 36℃ for 24 hr. The media for this experiment was employed after centrifugation at 8,000 rpm for 30 min and the pellet removed.

After culturing, 1-mL phage solution was added to 700 mL sterile water and stirred well. This solution was poured into the denture cleaner tank. A 0.1-mL sample was withdrawn before operation of the cleaner. Thereafter, over a period of 60 min, a 0.1-mL sample was withdrawn every 10 min, for a total of 6 samples.

Nutrient agar medium (10 mL) was sterilized, cooled to about 50℃, coagulated horizontally on sterilized petri dishes (9 cm×1.5 cm), and inoculated with 1-mL *E. coli* solution and sample solution, after which a further 10 mL of semi-solid agar was added. After the agar medium had set, the petri dishes were shaken, and were then left to stand at 36℃ for 24 hr. The number of phages was then calculated from the number of plaques on the surface of the medium. All the experiments were repeated 3 times and the results were statistically compared by averages and standard division.

RESULTS

The results of bactericidal effectiveness are shown in Fig. 2. In the experiment conducted with the ozone supply turned on, the initial number of bacteria was 3.1×10^3 CFU/mL. The number of bacteria fell to 1.0×10^0 CFU/mL after 10 min, and thereafter was 1.0×10^0 CFU/mL or less. In contrast, in the experiment in which the supply of ozone was cut off (air bubble only), the initial number of bacteria was 3.4 $\times10^3$ CFU/mL, and the number of bacteria had fallen to 3.0×10^3 CFU/mL 60 min

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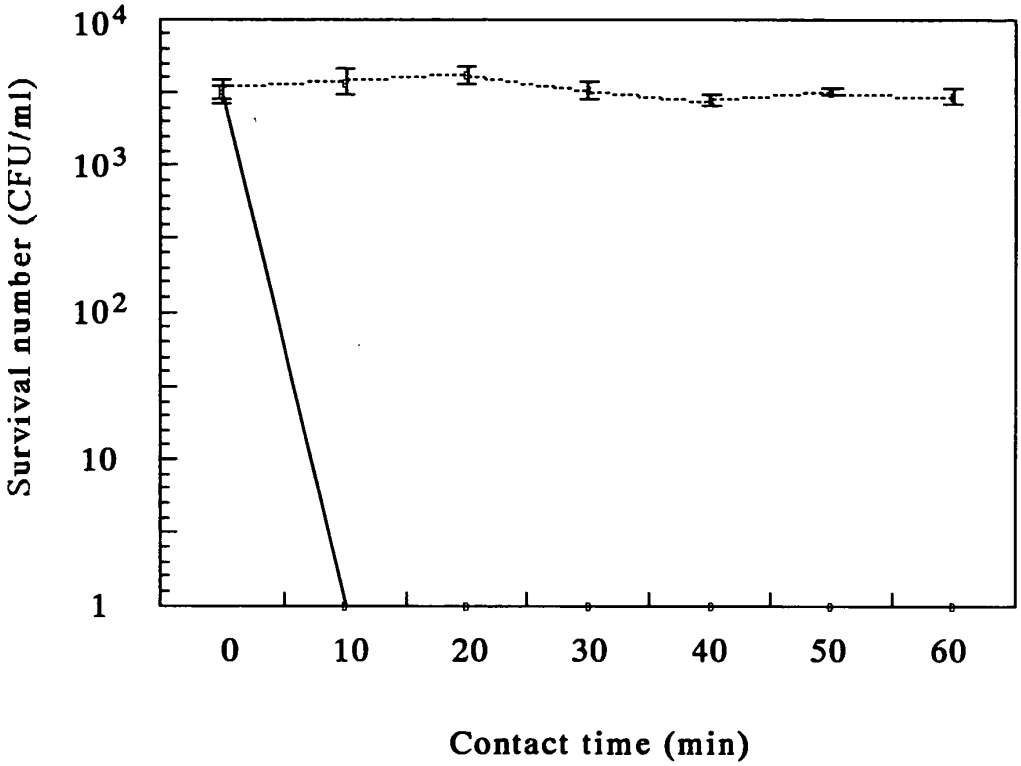

Fig. 2 Survival curves of MRSA by ozonation and airation.
—— Ozonation
····· Airation

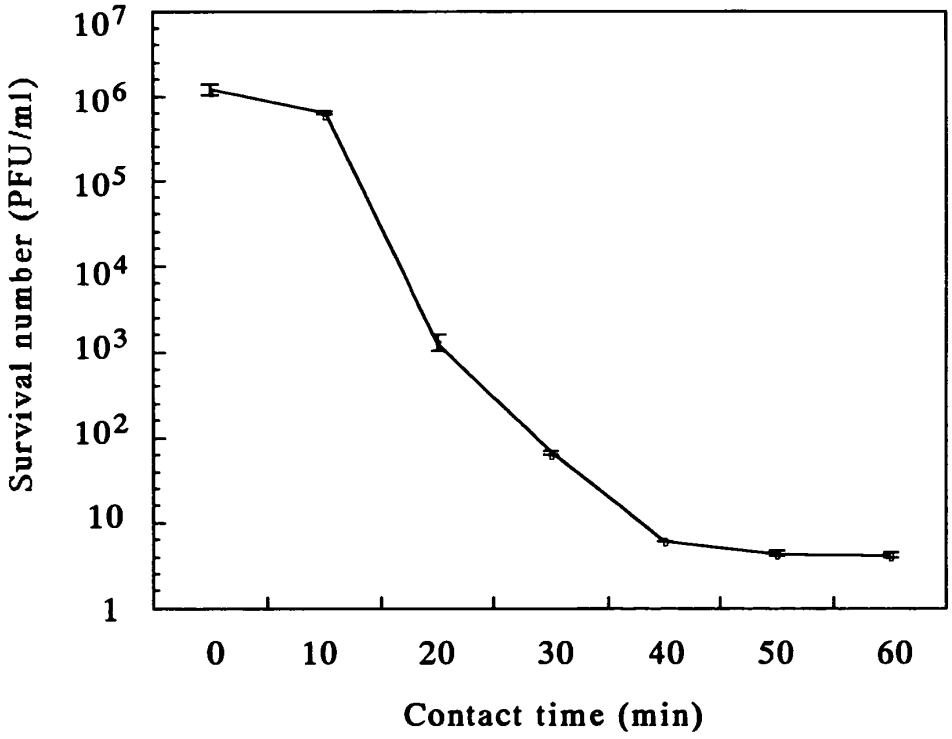

Fig. 3 Survival curve of T1 Phage by ozonation.

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later (no statistically significant difference).

The results of virucidal effectiveness are shown in Fig. 3. The number of phages was 1.2×10^6 PFU/mL before tozone treatment, fell to about 1/10 of that number 10 min later, and was 6.1×10^0 PFU/mL 40 min later.

DISCUSSION

Bacterial infection

The fact that MRSA has recently been detected in hospitals throughout Japan indicates that hospital infection has become an extremely serious problem[5]. Indications are that, originally, the most serious *Staphylococcus* infectious diseases affected children[5]. MRSA may be found in the sinuses, the pharynx and sites of skin erosion[5,6]. It proliferates in environments of comparatively high temperature and humidity, such as the perineal region. However, of late, hospital infection by MRSA has become a concern in those institutions that accommodate a large number of the bedridden elderly, arising from difficulties experienced in keeping such long-term patients clean[6–8]. An important contributory factor is the differences in the means of nutrition between these patients and healthy people; swallowing movements tend not to be sufficient, so nutrition tubes and endotracheal tubes have to be inserted. Staphylococci accumulate readily in such situations. In many cases, the liberal use of antibiotics to treat *Staphylococcus* infectious diseases has contributed to infection by MRSA. Patients whose stomatognathic function declines cannot perform sufficient masticatory and swallowing movements. In such cases, food debris remains in the oral cavity, and this results in an environment suitable for the increase of *Staphylococcus*. Furthermore, many elderly people wear removable dentures. The hands of care workers and co-dental staff who clean elderly people's dentures or teeth may come into contact with dentures and the washbasins in which dentures and toothbrushes are washed, and this is an important factor in the contraction of infection from dentures[9]. This underscores the value of bactericidal effectiveness against MRSA of the ozone denture cleaner we have developed. Ozone is a strong oxidizer, and its oxidizing action makes it an effective disinfectant. Presently, there are 2 main theories concerning the mechanism by which bacteria are inactivated by ozone. One theory is that bacteriolysis occurs because ozone destroys the cell wall and cytoplasmic membrane of the bacteria, as reported by Scott *et al.*[10] and Nebel[11]. The other theory is that the bacteria are rendered inactive without the occurrence of bacteriolysis, as indicated by some reports[12–15]. In the latter scenario, inactivated bacterial cells maintain a nearly normal shape, and inactivation is caused by outflow of the cells' constituent elements as a result of damage to the cell wall, irreversible obstruction, constituent changes in the cytoplasm[12], or inactivation of nucleic acid[13,14]. Uchiyama *et al.*[16] observed morphological changes in *Staphylococcus aureus* after ultraviolet irradiation or ozone treatment. The level of potassium (K^+) within a cell can be used to calculate the degree of damage to the cell's membrane; a fall in the amount of K^+ indicates damage. The results obtained indicate a decrease in

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the level of K^+ after ozone treatment. If ozone causes irreversible structural damage, it should be equally effective against common bacteria and resistant bacteria.

In this study, the number of bacteria decreased from 3.1×10^3 CFU/mL to 1.0×10^0 CFU/mL after treatment with about 10 ppm of ozone for 10 min in water. Ozone therefore appears to be an effective means of sterilization of dentures and toothbrushes, which are elements in the infection pathway. Tawara *et al.*[17] has reported the effectiveness of denture cleanser against MRSA. The cleanser resulted in the complete elimination of MRSA in 10 min, as did our results.

Viral infection

Various viral infections are encountered in the field of dentistry because viral infections are increasing rapidly. In particular, it is thought that hepatitis B and C viruses and HIV (the AIDS virus; acquired immunodeficiency syndrome, a typicaly slow virus infection), all of which cause extremely serious disease once infection has occurred, do not take hold easily without direct contact with bodily substances such as blood. The rate of infection of such viruses is increased by conditions within the oral cavity such as inflammation or open wounds that increase the chance of contact with blood. If the proper precautions against infection are neglected during dental treatment, the saliva or blood of a patient could contact with the dentist's face, and infection could occur via entry through the eyes or nose[5]. Furthermore, during the grinding of dentures, a fragment of the denture resin could fly off and lodge in the dentist's eye.

The dentures and toothbrushes to which a patient's blood can adhere may prove to be a very important source of infection, not only for dentists but also for other co-dental staff or the patient's family. That is to say, when family members or co-dental staff clean dentures or toothbrushes to which virus-infected blood has adhered, there is an increased chance of infection if they have preexisting cuts on their fingers or suffer cuts when the rubber gloves they wear to clean the dentures are accidentally torn. However, a denture sterilization method for viral infected patients has not yet been reported.

It is well known that ozone inactivates certain viruses at lower concentrations than chlorine[16,18]. Although the mechanism of the ozone action is still unclear, Shinriki *et al.*[19,20] reported that damage to a virus's protein coat and damage to its nucleic acid are both involved in the inactivation by ozone. It is generally assumed that damage to the protein coat impairs entry into a host and causes exudation of nucleic acid.

Our machine uses an ozone bubble system, and requires some 30 to 40 min to achieve inactivation of the virus. However, if a higher concentration of ozone is employed, this machine requires less time to inactivate the poliomyelitis virus. Also, daily sterilization of a patient's dentures and toothbrush should decrease the chance of infection.

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CONCLUSIONS

In this evaluation of the bactericidal effect of ozone in water against MRSA, using an ozone denture cleaner, the number of bacteria decreased from 3.1×10^3 CFU/mL to 1.0×10^0 CFU/mL after exposure to about 10 ppm of ozone for 10 min in water.

In this investigation of the effects of ozone on T1 phage, viral inactivation took place within 30 to 40 min.

There is no doubt that daily ozone sterilization of the dentures and toothbrushes of infected patients will dramatically lower the chance of infecting others.

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