Disinfection of Removable Dentures Using Ozone

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Over time, removable dentures tend to become unsanitary and emit unpleasant odors, and oral mucosa sometimes becomes inflamed or denture stomatitis is caused by denture plaque. Recently, various cleaning products designed to keep removable dentures sanitary have appeared on the market. It is known that denture plaque is mainly composed of *Candida albicans* (*C. albicans*), and that ozone seems to inhibit these micro-organisms. Accordingly, a denture cleaner using ozone bubbles (ozone concentration of about 10ppm) was considered as clinically appropriate because of its strong disinfecting and deodorizing power, and high biological safeness. The effectiveness of this cleaner against *C. albicans* was investigated using. Results showed that *C. albicans* decreased to about 1/10 after 30 min and to 1/10³ after 60 min.

Key words: Ozone, Denture cleaner, *Candida albicans*

INTRODUCTION

The use of dentures without proper hygienic care will in the long term cause denture stomatitis or the unpleasant odors. Mouthpieces and splints will also emit a characteristic odor. Smudges, staining and pigmentation of prostheses are produced not only by food remnants but also by micro-organisms in the oral cavity. Denture plaque accumulating on the mucosal surface of the denture largely consists of such eumycetes as *C. albicans*, as demonstrated by many researchers.¹⁻⁵⁾ The necessity of removing plaque from dentures is also important.^{6,7)} A denture cleaner utilizing the effects of ozone was manufactured because ozone has high biological safeness, and strong disinfecting and deodorizing power.⁸⁾ In addition, it rapidly decomposes, leaving no toxic residue.

The present paper evaluates the denture cleaner together with presentation of the results the disinfectant effectiveness of ozone against *C. albicans*.

MATERIALS AND METHODS

Outline of denture cleaner using ozone

The denture cleaner mainly consists of a power source, air pump, ozonizer and water tank to hold the dentures.(Fig. 1) Our previous report⁸⁾ described in detail the apparatus. Ozone

Fig. 1 Trial denture cleaner using ozone

concentration is about 10ppm in the ozonizer. The measurement of ozone concentration was performed by an ozone-concentration measurement device* at room temperature of 25°C and humidity of 60%. An experiment to determine the disinfectant effectiveness of this apparatus was performed using *C.albicans* (IFO1594).

Experiment condition

Two trial ozonizers (DC-1, DC-2) which generate ozone and one DC-0 which generates only air were used. The changes in the numbers of *C. albicans* in a concentration of 10ppm after 30 min and 60 min and in air alone (DC-0) after 60 min were examined.

Methods

1. Adjustment of culture medium

The composition of the culture medium is shown in Table 1. The medium was adjusted in a triangular flask, and ion-exchanged water (1 ℓ) was added, shaken and adjusted to pH 6.2~6.3. High-pressure sterilization was employed. After sterilization, the medium was cooled to 50°C and allowed to set for use as a plate medium.

2. Culture of *C. albicans*

C. albicans was painted on a plate medium by means of a platinum loop and cultured at 25°C for 72 hours.

3. Preparation of cell-suspension

The propagated *C. albicans* on the plate medium was collected by platinum loop, and suspended in sterile water (10ml) with a turbidity of about 0.4 at 420nm using a spectrophotometer. This suspension (2~3ml) was added to sterile water (2 ℓ) and mixed thorough-

* OZM-G21-2W, Okitronics Co. Ltd., Tokyo, Japan

Table 1 Composition of culture medium

Pepton	5
Yeast extract	3
Malt extract	3
Glucose	10
Agar	15

(g)

ly. This was to serve as the test sample solution.

4. Protocol

- (1) Test sample solution was poured into the tank of the test apparatus (DC-0, 1, 2).
- (2) Test sample solution was stirred thoroughly, and 0.1ml of the solution was removed, and the test apparatus put into motion.
- (3) 0.1ml was added to 0.9ml of sterile physiological salt solution to make 10-fold dilutions of the sample solution.
- (4) 0.1ml of this diluted solution was added to a 0.9ml sterile physiological salt solution diluted 10-fold for making a solution diluted 100-fold.
- (5) 0.1ml of 10-fold and 100-fold dilutions of the test sample solution were placed on the plate media and cultured at 25°C for 72 hours.
- (6) After the apparatus was set in motion for the appointed test period, the numbers of bacteria were measured following the procedure outlined in sections (3)~(5) culturing *C. albicans* in 10-fold and 100-fold dilutions. In addition, 0.1ml of the test sample was also placed on a plate media and cultured.
- (7) After culturing, the number of supplied bacillus colonies on each plate was counted, and the number of *C. albicans* in 1ml of solution was calculated adopting the number of the dilute ratio in the range of 30~300.

RESULTS

The findings of the present study on the disinfectant effectiveness of an ozone concentration of 10ppm against *C. albicans* are as follows (Table 2, Fig. 2):

In DC-1, the initial number of *C. albicans* at 4.85×10^3 CFU/ml had decreased after 30 min to 1.31×10^3 CFU/ml and after 60 min to 1.8×10^0 CFU/ml. In DC-2, the initial number of *C. albicans* was 4.10×10^3 CFU/ml; after 30 min the number had decreased to 8.45×10^2 CFU/ml and after 60 min to 1.5×10^0 CFU/ml. These figures were similar to those of DC-1. In air only, the initial number of *C. albicans* was 4.35×10^3 CFU/ml; after 60 min, the number had decreased to 3.80×10^3 CFU/ml, a decrease limited only to about 13% of the earlier reading.

Table 2 Effect of ozone on survival of *C. albicans*

Code	Motionless	After 30 min	After 60 min
DC-0 (Air only)	4.35×10^3	—	3.80×10^3
DC-1 (O ₃ 10ppm)	4.85×10^3	1.31×10^3	1.8×10^0
DC-2 (O ₃ 10ppm)	4.10×10^3	8.54×10^2	1.5×10^0

(CFU/ml)

Fig. 2 Survival of *C. albicans*

DISCUSSION

Disinfectant effect against C. albicans

A denture cleaner utilizing ozone which possesses powerful disinfectant and deodorizing effectiveness has been manufactured. Research was conducted on its effectiveness against *C. albicans* and as a result it was found that *C. albicans* decreased to about 1/10 after 30 min and to 1/10³ after 60 min. Naito⁹⁾ reported on the notable differences of resistance in some 300 different species of fungi and bacteria after ozone treatment in the disinfection of foodstuffs. He pointed out that ozone showed its most intensive disinfectant power against *Lactic acid bacterium*, followed by yeast fungi, yeast fungi spores, *Penicillium* spores, *Aspergillus* spores and *Bacillus* spores. Among these fungi, ozone showed the highest effectiveness against the *Candida* genus. Some reports¹⁰⁻¹²⁾ have indicated that ozone in fluid has proved effective as a disinfectant against *C. paracreus*, *C. tropicalis*, *C. kurusei*, *C. tropealis* and *C. utilis* from the *Candida* genus, but there are no reports concerning its effect on *C. albicans*, the main constituent bacterium of denture plaque. In our experimental denture cleaner (ozone concentration about 10ppm), the number of *C. albicans* decreased to 1/10 after 30 min., and to 1/10³ after 60 min, but eliminating the bacterium entirely seems to be impossible. The problem is related to an assessing procedure to which level, disinfection should be maintained. Previous reports have mainly focused on ozone's practical usage rather than establishing the basic mechanism of ozone disinfection. However, studies of the biological effect of ozone on

the cell wall or the cell membrane and various enzymes needs to be continued in the future.

Ozone utilization

Since ozone is an unstable gas molecule, its production involves intense oxidation and emission of oxygen. The oxidizing power of ozone ranks second to that of fluorine. The chemical behavior of ozone has led to its being employed for deodorization, decolorization, disinfection, and decomposition of organic matter. The disinfectant effectiveness of ozone against viruses and bacilli was recognized a long time ago and investigations were carried out in waterworks, sewage, air, food, and in the medical field. The merits of ozone are that (1) it can be generated from air or water by means of electricity, (2) it is easy to control the amount generated, (3) there is no secondary environmental pollution because ozone readily decomposes, (4) there is only a very slight possibility of the generation of toxic substances. There are disadvantages however, in that ozone is (1) unstorable, (2) allows no osmosis by disinfection, (3) Quickly dissipates and (4) is difficult to handle because of its strong reaction. Denture cleaning products are required to be cost effective, leave no toxic substance after decomposing as well as having high biological safeness, and to possess extensive antibacterial and deodorant effects. Especially the biological safeness of denture cleaners are important, although mishaps with denture cleansers have not yet been reported in Japan. However, in the U. S. A, about 150 cases of misuse or accidental drinking of cleansers are reported^{13,14)} by NCPCC (National Clearinghouse for Poison Control Center) annually. Taking every factor mentioned above into consideration, ozone's remarkable disinfectant and deodorant power, its short half-life in water, its swift decomposition and the ease of disposing of any effluent make it suitable for cleaning dentures.

CONCLUSION

A denture cleaner utilizing ozone which possesses powerful disinfectant and deodorizing effectiveness has been manufactured. Research was conducted on its effectiveness against *C. albicans* and as a result it was found that *C. albicans* decreased to about 1/10 after 30 min and to 1/10³ after 60 min. Consequently, with further improvements to this denture cleaner, it should be possible to utilize ozone for the cleaning and disinfecting of removable prostheses.

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でも偏析が確認された。この結果は、作用した遠心力の強大なことを裏付けている。しかしながら、チタン合金、Ti-6 Al-4 V を鑄込んだ場合、遠心力によるマクロ偏析

は認められなかった。以上の結果より、今回使用した遠心鑄造機では強大な遠心力が発生するが、この合金にマクロ偏析を生じさせることはない、と結論された。

オゾンを利用した可撤性義歯の殺菌

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口腔内には多くの細菌が棲息しているため、可撤性義歯を衛生管理が十分に行われていない状態で、長期間使用しているとデンチャープラークが義歯表面に付着し、義歯性口内炎を起こしたり、義歯特有の臭いを発するようになる。そのため、可撤性義歯を可及的清潔に保つことが重要である。

そこで、われわれは殺菌力、脱臭力に優れたオゾンに

着目し、これを義歯の殺菌、洗浄に使用するため、オゾンを使用した義歯洗浄器を試作した。今回はこの試作洗浄器を使用し、デンチャープラークの主体である *Candida albicans* に対する殺菌力を調べた結果、約 10 ppm のオゾン濃度では、30 分後に約 1/10、60 分後に 1/10³ 程度に減少することが判明し、可撤性義歯の洗浄に使用可能と思われた。

ヒトおよび動物皮膚に対するデンティンプライマーとしての 2 種ダイオール水溶液の安全性の評価

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この研究の目的は、当教室で開発されたデンティンプライマーすなわち 62.5%ethylene glycol, 45% 1, 6-hexanediol 水溶液の皮膚安全性を一次皮膚刺激試験、累積皮膚刺激試験、Guinea Pig Maximization test およびヒトパッチテストによって検討、観察することである。その結果、一次皮膚刺激試験、累積皮膚刺激試験とヒトパッチテストでは両水溶液ともに刺激性は認められな

かった。しかしながら、Guinea Pig Maximization test による 2-HEMA に感作された動物に対し、100%hexanediol で弱い誘発反応が認められた。以上の結果より、2-HEMA の様なメタクリル酸プライマーと比較してethylene glycol や 45% 1, 6-hexanediol 水溶液は比較的安全で臨床的にも十分使用可能なプライマーであると思われる。