TRANSLATION

Nihon University Oral Science 45: 105-109, 2019

Contamination Prevention for Dental Unit Water Systems Using Nano Bubble Water

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Key Words：dentalunit, water system, nano bubble water, contamination
Nihon Univ. J. Oral Sci. 45：105～109, 2019

INTRODUCTION

In recent years, microbial contamination of the water supply system in dental chair units (hereinafter referred to as "units") has been reported in newspapers and other media[1)] and has been viewed as a concern. It has been reported that microorganisms such as bacteria, fungi, and protozoa can settle in the unit's water supply lines (plastic tubes used to supply water to high-speed turbines, three-way syringes, etc.) [2-7)].

These microorganisms form biofilms, which are also known to be responsible for increasing the number of free-floating microorganisms in water. Although most of the microorganisms recovered from water supply systems are heterotrophic aquatic bacteria with low pathogenicity[8, 9)], various oral bacteria, Pseudomonas aeruginosa, and Legionella spp. have also been isolated[10, 11)], suggesting an increased risk of opportunistic infection in infants, the elderly, and those with weakened immune systems. In other countries, there have been reports of deaths due to Legionella infection following dental treatment in the elderly [12)].

In general, the use of microfilters, chlorine, backflow prevention measures during turbine shutdown, and flushing (water drainage) before treatment are used to prevent contamination of the water supply system. Although some reports suggest that the influx of new microorganisms can be controlled by the use of microfilters[13)], the Centers for Disease Control and Prevention (CDC) reported that the use of chemical disinfectants in addition to filter attachment and flushing after usage is necessary for biofilm removal and inactivation in the dental unit water supply circuit[14)].

Although the use of chlorine can disinfect if it is in a free state, dichlorination occurs during the closing hours and on days when the clinic is closed, and some microorganisms may be able to live in the clinic. Furthermore, the use of high concentrations may affect the human body, and there are concerns about increased risks of the development of resistant bacteria. In addition, it is difficult to remove biofilm by flushing[15)]. Since the unit water is likely to be contaminated by biofilm formation in the water supply line due to long-term continuous use of the unit, it is necessary to take immediate measures to prevent biofilm formation in the water supply system (tubing).

Nanobubbles are generally ultrafine bubbles with diameters of 200 nm or less. The cleaning effect of bubbles has been known for a long time. Nanobubbles float extremely slowly and exist for a long time, indicating high penetration into biofilm, detaching effect, and cleaning action. This is one of

the proofs that nanobubbles have attracted attention in the medical, food processing, and livestock industries, and have been applied in various fields.[16, 17]. In the dental field, nanobubbles have been utilized for cleaning unit water supply systems.

In this study, a dental chair unit equipped with a nanobubble generator has been utilized in daily practice to evaluate the effect of nanobubble water.

METHOD

1. Nanobubble Generator

Dr. Nano for Dental (outer diameter 25 mm × length 70 mm, manufactured by New Environmental Technology Council, Tokyo, Japan) (hereinafter referred to as "Dr. Nano") was attached to the water pipe in the junction box of the unit.

2. Period

The period was one month from February to March 2018.

3. Comparison of bacteria counts in unit discharge water with and without nanobubble water application

1) Method

One month after the Dr. Nano was installed in units (made by companies X, Y, and Z) of 8 (A to H) dental clinics (10 units in total) (hereinafter referred to as the "test group"), the water discharged from the three-way syringe of the installed side was sampled and the bacteria count was measured. The control group consisted of 10 units without Dr. Nano in each dental clinic (hereafter referred to as "control group"), and water samples were collected and measured in the same manner as the test group. Each dental clinic had one unit for the test and control groups, except clinic A which had three units for each group. During the experimental period, normal dental care was provided.

2) Bacterial count

Dental EZ-Dip ® (Sanai Oil Co., Ltd., Tokyo, Japan), which is commercially available, was used to measure the number of bacteria. After 48 hours of incubation, the bacterial counts were compared with the control chart provided with the Dental EZDip ® and determined. The control chart is classified into six levels of viable counts (CFU/ml) ranging from 10^1 to 10^6.

(cf.; Dental EZ-Dip®, https://www.dental-ez-dip.com/example/).

3) Statistical analysis

Statistical software SPSS20.0J (IBM) was used to compare the number of bacteria (ranks) detected in the units with and without nanobubble water application by Mann-Whitney U test. The significance level was set at 5 % or less.

RESULTS

Bacteria were detected at 0 CFU/ml in all 10 units in the test group. In contrast, of the total 10 units observed in the control group; one unit had 0 CFU/ml, two units had 10 CFU/ml, two units had 10^2 CFU/ml, three units had 10^3 CFU/ml, one unit had 10^4 CFU/ml, and one unit had 10^5 CFU/ml. The number of bacteria in the test group was significantly ($p < 0.001$) lower than that in the control group (Table 1, Fig. 1).

Table 1 Number of total viable bacterial rank by the Dr. Nano with or without.

(CFU/ml)

Dental Office	Dental unit manufacturer	With Dr.Nano	Without Dr.Nano
A-1	X	0	10^4
A-2	X	0	10^5
A-3	X	0	10^3
B	Y	0	0
C	Y	0	10^2
D	Z	0	10^1
E	Z	0	10^3
F	X	0	10^1
G	Y	0	10^3
H	X	0	10^2
			$p < 0.001$[a]

a: Comparison between Dr. Nano with and number of bacteria rank of without, Mann-Whitney U test

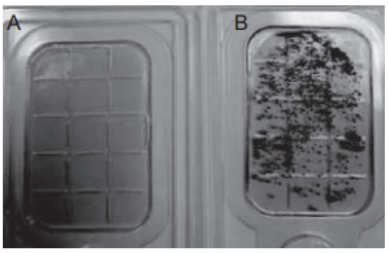

Fig. 1 Culture result by the EZ-Dip ®
A; with Dr. Nano, B; without Dr. Nano (Dental Office A-1)

DISCUSSION

Detection of bacteria have been reported in dental units globally[-8)], and the results of study indicate that a large number of bacteria were detected in the water discharged from the units. The increase

in bacteria in the water supply system can be attributed to the backflow of bacteria due to the sackback (retraction) phenomenon, the complexity of the piping structure of the unit, stagnation of water flow due to stoppage at night or on holidays[18], and the presence of biofilm formed inside the tubes of the water supply system.

In the units equipped with Dr. Nano generate nanobubbles, by tap water pressure hits a screw for a short time, causing cavitation, and the air dissolved in the tap water is deposited as nanobubbles. The advantage of Dr. Nano is that it is smaller than other nanobubble generators and can be easily attached to and detached from the water pipe installed in the junction box of the unit, without the need for electrical work. The generated water shows no color change nor change in taste or odor. Since no chemical bactericidal action is observed, the physical action of the nano-level bubbles is considered to be the main factor in the inhibition of biofilm formation and detachment.

With the application of nanobubble water (after Dr. Nano was installed), a large number of heterotrophic bacteria exceeding the target value (2×10^3 CFU/ml or less)[19] for water quality control were detected for approximately 10 days, but after approximately one month, the number decreased to below the target value (unpublished data). This is thought to be a result of the fact that it took at least 10 days for the biofilm already formed in the water supply system due to the fact that the subject unit had been used for several years for medical treatment to be peeled off by the nanobubble water. Therefore, it is necessary to sufficiently remove the biofilm by flushing for about 10 days after installation of Dr. Nano. Furthermore, after the biofilm was detached from the water supply system, it was suggested that the nanobubble water acted to inhibit the formation of the biofilm. Since nanobubbles float extremely slowly, they exist in the tubes of the water supply system even when the water flow is stopped at night or on holidays, and it is assumed that they inhibit the formation of biofilm.

As the detection of bacteria in dental units has become a problem and efforts are being made to control water quality and implement countermeasures in dental clinics, the Dental EZ-Dip® Microbiology Simplified Measuring Kit has been developed as a simple water quality testing method. This kit allows specimens to be collected on a plastic tray medium and tested at 37 °C for 24 hours or longer, and the number of viable microorganisms is measured by comparison with a control table, providing test results equivalent to those obtained by general testing methods. The Dental EZ-Dip® is useful as a water quality test in dental clinics because it can be performed quickly and easily.

A comparison of the number of bacteria in the effluent from the units in the test group (equipped with Dr. Nano) and the control group (not equipped with Dr. Nano) showed that no bacteria were detected in the test group. On the other hand, bacteria were detected in 9 out of 10 units in the control group. In addition, five of the nine units in the control group were found to have bacteria counts of 1×10^2 CFU/ml or higher, the water quality standard for general bacteria. Imazato et al.[20] compared bacterial counts between polyurethane tubing and flexible fluorine-coated tubing as a means of preventing contamination of unit water supply systems and reported that although the polyurethane tubing had a higher bacterial count, a large number of bacteria could be detected even in the flexible fluorine-coated system. In this study, there were three unit manufacturers that installed Dr. Nano, and the materials used in the water supply system were not specifically examined. Although we did not uniformly examine the number of years the units had been in use or the nature of the treatment in each dental clinic, we confirmed the effectiveness of the application of nanobubble water for one month even under the different conditions. The reason why bacteria were not detected in one unit in the control group could be due to the small number of years the unit had been in use, the relatively high frequency of treatment, etc. However, we did not examine this issue in detail.

Although it is mentioned that special material piping specifications should be used for slightly acidic electrolyzed water (effective chlorine concentration 10 to 30 ppm, pH 6.3 to 6.8)[21] and the introduction of residual chlorine correction disinfection systems[22], there is no evidence that the application of nanobubble water will have any effect on the unit itself. Since nanobubbles do not change the properties of water, they do not cause changes in pH, and the possibility of corrosion of the water supply system or elution of metals is extremely low, so they are considered to be safely operated. However, considering the ability of biofilm formation, it would be more effective to

select tubing for the water supply system that is made of a material with a smooth inner surface.

These results suggest that the application of nanobubbles to dental units may play a useful role in the detachment and inhibition of biofilm formation. However, the study period was short (one month) and bacteria were only detected at a single point in time. In order to measure specific heterotrophic bacteria and effects on piping, longer term observations of bacteria may be necessary. In addition, the Japanese Industrial Standard (JIS) "Dentistry - Test Methods for Biofilm Treatment in Water Supply Pipelines of Dental Units "[23] has been presented regarding the inhibition of biofilm formation and detachment in units, and further detailed studies in accordance with these standards are considered necessary.

CONCLUSIONS

The application of nanobubble water to dental units is effective in preventing water contamination in the water supply system.

ACKNOWLEDGEMENTS and CONFLICT of INTEREST

This research was partially funded by a joint research grant from the New Environmental Technology Council. We would like to express our sincere gratitude to Mr. Hiroyuki Kawai, Mr. Masahiko Tanaka, and Mr. Satoshi Nagashima of the New Environmental Technology Council, Dr. Takahiro Otsu of Opian Dental Office, and Dr. Osamu Yamazaki of Harajuku Dental Office for their advice and support throughout the conduct of this research.

The presenters have no COI relationships with any companies or organizations that should be disclosed in connection with this paper.

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