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THESIS APPROVAL PAGE FOR MASTER OF SCIENCE IN ORAL BIOLOGY

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The effects of using ozone treated water on biofilm accumulation in dental unit water lines.

Introduction

The control of bacterial contamination in dental unit water has been a focus of public health for many decades. It has long been established that water dispensed from dental units can potentially contain a greater density of bacteria than would be suitable for municipal drinking water (1, 2). The many areas of small diameter tubing in dental units provide a suitable environment for the formation of biofilm, which can then lead to greater levels of planktonic bacteria in the water dispensed and used during patient care (3). While the majority of the bacteria found in these biofilms are considered benign, and are typical of those usually found in potable water, known pathogenic species such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Legionella* sp. have been isolated from dental units (2, 4, 5). This poses a potential threat to public health, and to dental healthcare workers who are routinely exposed to aerosols produced from dental unit water (2, 4, 5). While this concern is in many respects theoretical, there have been prominent cases where contaminated dental unit water has been suspected in causing infection in dental patients and dental professionals (2, 4, 6, 7). As a result of these findings, there is now great public pressure on the dental profession to ensure that the bacterial exposure to patients and dental professionals is minimized. Current recommendations indicate that water sourced from dental units should exhibit bacterial counts consistent with existing standards for potable drinking water (2).

Strategies for minimizing bacterial contamination of dental unit water have focused on three main areas (8). One area of development in recent decades has been improved design of dental chairs with internal components intended to reduce the potential for biofilm formation. Another area of focus involves periodic use of chemical treatments to eradicate or reduce existing biofilm within dental unit water lines. Lastly, control of the quality and bacterial contamination of water entering dental units has become a key area of focus (8, 9). The control of water quality before entering the dental unit can minimize the number and diversity of bacterial species that are introduced to the water lines. This may reduce biofilm formation, and in turn reduce the bacterial load of water that is released from the units during patient care.

The powerful oxidative capacity of ozone has demonstrated success as a means of controlling a variety of pathogens in water treatment, and food processing (10, 11, 12, 13). The ozone molecule exists in a chemically unstable configuration, and will readily degrade to form oxygen (O₂) and a single oxygen free radical species (13). The free radical that is produced has a potent ability to target bacteria, fungi, and viruses (13). In addition to well-established uses in treatment of potable water, waste water, and food processing, ozone has more recently been proposed as a useful sterilization tool in healthcare settings (14, 15). This study will attempt to demonstrate whether use of ozone to treat water supplying dental units can reduce the bacterial

counts in water emitted from these units during patient care. The use of ozone treatment will be compared to two other treatment methods, tap water and filtered water.

Infection control in military dental facilities has been an area of increasing focus in recent years. Implementing effective infection control measures that are also economical and efficient is of great importance. Ozone treatment of dental unit water may contribute to proper infection control in military dental clinics. If this measure is found to be effective at reducing bacterial load in dental unit water lines, this technique may also have unique value in remote military treatment settings or humanitarian missions where other means of controlling water quality are not readily available.

Materials and Methods

Three dental chairs located in the periodontics clinic at the Air Force Post Graduate Dental School, Joint Base San Antonio (JBSA) – Lackland, San Antonio, Texas, were utilized to determine differences in bacterial load in dental unit water between units supplied using three different sources. The water source of one dental chair was replenished throughout the study using regular tap water sourced from a sink adjacent to the dental operator. A second chair had its water supply refilled using water sourced from an in clinic water filtration system (Isopure Water ISO-RO6ALK, IPW Industries, Santa Ana, CA, USA). The third dental chair had its water source replenished with ozonated tap water. The tap water was again sourced from a sink adjacent to the operator. Ozonation of the tap water was accomplished using an Aqua-8 Ozone Generator (A2Z Ozone, Louisville, KY, USA), and was completed just prior to refilling of the water source. Air dispensed from the unit through a length of plastic tubing was forced through a diffuser stone, which allowed ozone to be introduced to the tap water. This was done for 20 minutes each time that the dental unit water supply had to be refilled. The water was then immediately placed in the dental chair's water supply.

The study took place over a 90 day period, which corresponded to the planned interval between bacterial testing for all dental chairs in the clinic. At the beginning of the study, water sourced from all three of the dental units was tested using disposable testing kits (Merck KGaA, Darmstadt, Germany). After 48 hours of incubation at room temperature, the test kits were evaluated under 2.5x magnification. The colonies present on each test kit were counted to determine the number of colony forming units per mL of water sourced from each of the three dental chairs. The initial CFU values were compared to ensure that each of the three chairs began the study with a similar bacterial load. The same disposable testing kits were also used at the beginning of the study to evaluate each of the three water sources planned for use.

All three dental units were not being utilized for patient care during the study period. To simulate regular daily use of the dental chairs, the air water syringe of each unit was operated continuously for five minutes, once per day on each day that the clinic was treating patients. Planned maintenance of each unit called for treatment with a diluted bleach solution every two weeks. This treatment was not completed during the study period in order to better highlight differences in bacterial count relating to the use of different water sources. At the conclusion of

the 90 day period, water emitted from the three way syringes of all three units was tested again using the same protocol utilized at the beginning of the study. The CFU values for water emitted from each of the chairs were compared to determine differences related to the three water sources.

Results

The initial CFU values for the three dental chairs utilized are displayed in table 1. All three of the dental chairs had been chemically treated within a few days prior to beginning the study. The dental unit water from all three chairs produced a CFU count of 0 CFU/mL. No bacterial colonies were visible on any of the testing kits, indicating that all three chairs initially were free of bacteria in the water lines

Table 1

Chair Number	Initial CFU Count
Chair #1	0 CFU/mL
Chair #2	0 CFU/mL
Chair #3	0 CFU/mL

The initial test results for the three different water sources are displayed in table 2. The ozone treated tap water showed an initial CFU value of zero, with no bacterial colonies evident on the test kit. The filtered water sample showed an initial CFU value of 30/mL. The tap water sample showed approximately 300 CFU's per mL.

Table 2

Water Source	Initial Source CFU Count
Ozone Treated Tap Water	0 CFU/mL
Filtered Tap Water	30 CFU/mL
Tap Water	326 CFU/mL

The 90-day testing results for each of the three water sources are shown in table 3. They all remained similar to their initial values, with ozone water again showing zero CFU's. Filtered tap water remained low, and tap water remained the highest. The bacterial counts for all three water sources remained similar throughout the study period.

Table 3

Water Source	Final Source CFU Count
Ozone Treated Tap Water	0 CFU/mL
Filtered Tap Water	27 CFU/mL
Tap Water	310 CFU/mL

The final testing results for each of the three dental chairs are seen in table 4. Chair #1 was replenished throughout the study using ozone treated water. Chair #2 was replenished using filtered water. Chair #3 was refilled using unmodified tap water. The testing results for all three

chairs showed markedly increased bacterial counts from the study start, when all chairs were recording 0 CFU/mL. At 90 days, the use of different water sources demonstrated no difference, with each chair having a CFU count of greater than 500 CFU/mL. The actual counts had to be estimated due to the great density of colonization seen on the test kits.

Table 4

Chair Number/Water Source	Final Chair CFU Count
#1 Ozone Treated Tap Water	>500 CFU/mL
#2 Filtered Tap Water	>500 CFU/mL
#3 Tap Water	>500 CFU/mL

Discussion

This in vitro study has some basic key findings. First, it should be noted that all three water sources registered CFU values that were less than 500/mL, rendering all to be considered potentially reasonable sources for replenishment of dental chairs. It should also be noted that ozone treatment substantially reduced the CFU value of regular tap water. At both the study start, and at 90 days, ozone treated water demonstrated the lowest bacterial count, with untreated tap water showing values more than 300 CFU's greater than ozone. The in-clinic micro filtration system also substantially reduced the CFU value of tap water, although not as much as ozone treatment. Lastly, a key finding is that all dental chairs in this study exhibited similar bacterial levels following the 90 day period. This suggests that the three water sources did not have a major effect on bacterial count of water emitted from each chair. Eliminating the routine maintenance treatment of the dental chairs during the study could have contributed to the excessive amounts of bacteria in the dental unit water.

One of the clear limitations of this study is that it only dealt with three dental chairs. A larger number of chairs, with more frequent water line and source water testing may provide additional insight into this topic. The water testing schedule was also limited, with values recorded at study start, and at 90 days, but without verification testing throughout the study. Lastly, the ozone generator used in this study was a somewhat primitive unit. There was no way to verify calibration of the unit or its continued function as it was used throughout the study.

Conclusions

It can be concluded that ozone treatment considerably reduced bacterial contamination compared to both filtered tap water and ozone treated tap water. Despite the lower CFU count of ozone treated water, regardless of which water source was used to replenish the dental chairs, all experienced high bacterial counts considered unsafe for patient care at study conclusion. Lastly, it may be inferred that other water quality maintenance measures, such as the regular use of chemical agents, may be of greater value in reducing bacterial load. This topic will likely continue to receive attention, due to the increased focus on infection control standards in dental healthcare settings.

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