

A simulated-use evaluation of hydrogen peroxide disinfectant for preventing biofilm formation in dental unit waterlines.

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INTRODUCTION: The water obtained from dental units via syringes, air rotors and low-speed handpieces may be heavily contaminated with microorganisms and thus may be a potential source of infection for both practice staff and patients, particularly those who are medically compromised or immunocompromised. The range of microorganisms isolated includes both environmental organisms and opportunistic and true human pathogens. DUWL contamination is caused predominantly by bacteria which form multispecies adherent biofilms on the inside of the waterlines and provide a reservoir for continuous contamination of DUWL. The development of biofilms is facilitated by the ready adhesion of bacteria to the hydrophobic polymeric plastic tubing material used in dental equipment and by the low flow rate and the intermittent patterns of use of water with over-night and week-end stagnation. Numerous procedures for improving the water quality in DUWL implying prevention of biofilm formation or elimination of biofilms have been proposed but none has been universally adopted that is both efficient at eliminating biofilms, as well being safe for patients. Disinfection agents based on hydrogen peroxide are claimed to break up biofilms in DUWL. Using an in vitro biofilm reactor system which mimics the use of a dental unit and allows the development of a biofilm, the purpose of the present study was to evaluate the ability of one hydrogen-peroxide disinfectant (Oxygenal) to minimize biofilm formation in waterlines.

METHODS: The biofilm reactor system was made of 2 pieces of waterline tubing (PVC) filled with water that was alternately made to circulate or remain static by use of an electronic controller so as to simulate daily dental unit usage. The test period was 10 days with the daily (five-days-per-week) controller cycle. The test program was as follows: water circulated for 15 mn and remained static for 30 minutes in the waterline tubing between 9 a.m. and 12 noon and between 1.30 and 5.30 p.m. Stagnation of water was also observed between 12 noon and 1.30 p.m., over-night and during the week-end (periods of non-use). One of the pieces of waterline tubing was kept untreated and was filled with municipal water as a control while the other (test tubing) was filled with municipal water treated with Oxygenal (0.02%). According to the recommendations of the manufacturer, at day 5, a solution of

municipal water + Oxygenal (0.25%) was circulated in the waterline tubing for 30 mn and then this solution remained static in the tubing over the week end.

Bacterial analysis

Water samples from the pieces of tubing and from the municipal water source (1 ml) were analyzed in order to evaluate the bacterial contamination of waterline fluids and municipal water.

Biofilm samples were obtained as follows. External waterline tubing surfaces were wiped with a sterile alcohol wipe and 3 cm of the tubing was cut off with a presterilized lancet. Internal surfaces were rinsed with sterile water to remove loosely adherent cells.

Using a sterile microbrush, biofilm was scraped from the surface into 1 mL of sterile distilled water. Samples were spun for 3 mn in order to dissociate bacterial aggregates.

Total viable counts (TVC) were performed on decimal dilutions of the biofilm and the water samples. Samples of appropriate dilutions were plated onto R2A agar and incubated at 25 C° for 7 days and the number of colony forming units/cm² (biofilm sample) and the number of colony forming units/ml (water samples) were calculated.

Water and biofilm samples were analysed at day 0 and at day 10 since previous investigations had demonstrated that a mature biofilm covering the internal surface of tubing was obtained after 10 days.

SEM analysis. Sections of waterline tubing were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer for 30 mn at 4°C. Fixed samples were washed twice in 0.2 M cacodylate buffer followed by increasing concentrations of ethanol. After drying, samples were coated with gold-palladium and examined via SEM (Hitachi S800).

RESULTS: Table 1 shows the average number of cfu/mL (water samples) or cfu/cm² (biofilm samples) in the control and treated waterline tubings and in the municipal potable water source. Quantitative analysis of control waterline tubing revealed that the water and tubing surfaces were heavily populated with bacteria with an average bacterial count of 7.22x10⁴ cfu/ml and 8.44x10⁴ cfu/cm² respectively.

Treatment of the water by Oxygenal for 10 days considerably reduced bacterial density with an average count of 140.5/mL in water samples and in 0.50 ufc/cm² in biofilm samples

Table 1: Average bacterial density of aerobic heterotrophic bacteria in municipal water, water and biofilm samples.

	Day 0	Day 10 Control	Day 10 Oxygenal®
Municipal water source (ufc/mL)	182 (1.02)		
Water samples from tubing (ufc/mL)		7.22x10 ⁴ (0.95)	140.5 (13.44)
Biofilm samples (ufc/cm ²)		8.44 x10 ⁴ (1.37)	0.50 (0.71)

- Ranges are shown in parentheses

SEM analysis of the tubing surface obtained from the control waterline tubing revealed a dense biofilm matrix in every field of the luminal surface of the tubing (Fig.1).

SEM analysis of tubing obtained from the treated waterline tubing revealed the probable presence of a mineral matrix without bacteria (Fig. 2)

DISCUSSION & CONCLUSIONS: This study simulated conditions in dental unit waterline tubings over a period of 10 days, creating conditions that promoted growth of biofilms in dental tubing as demonstrated in untreated tubing. The untreated control developed extensive biofilm resulting in contaminated water at levels consistent with the range reported in many studies on the microbial water quality of dental units in actual clinical service.

Figure 1: SEM of control tubing lumen surface showing a dense biofilm matrix.

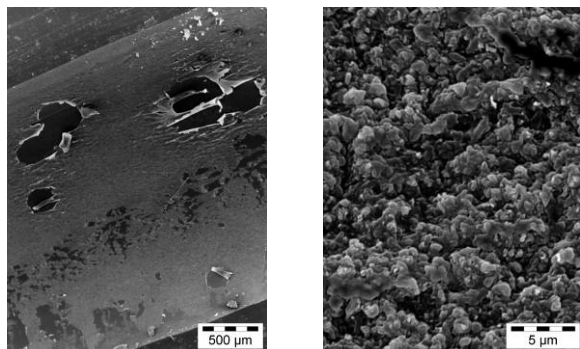
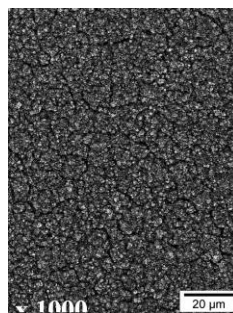


Figure 2: SEM of treated tubing (Oxygenal®) lumen surface showing a mineral matrix.



In this simulated study, the test product effectively controlled bacterial contamination in incoming water and prevented biofilm formation during daily use and over periods of inactivity. The standard (lower limit bacterial load < 200 cfu/mL) suggested by the American Dental Association for bacterial contamination of dental unit waterlines was achieved by using hydrogen peroxide continuously.

This laboratory test method is valuable for reproducibly assessing the efficacy of products aimed to control dental waterline contamination.

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