

Final Report
(LLUSD-RS-0402)

**Evaluation of the Effectiveness of Ultradent Sterilox on
Controlling Microbial Contamination, Removing Biofilm and
Reducing Endotoxins in Dental Unit Waterlines**

**Center for Dental Research
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January 14, 2005

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Title

Evaluation of the Effectiveness of Ultradent Sterilox on Controlling Microbial Contamination, Removing Biofilm and Reducing Endotoxins in Dental Unit Waterlines (DUWLs).

Sponsoring Agency

Ultradent Products, Inc.
505 West 10200 South
South Jordan, Utah 84095
Attention: Carol Jent

Conducting Agency

Research Services
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Loma Linda University School of Dentistry
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Study Number

LLUSD Research Service Study Number: LLUSD-RS-0402

Principal Investigator

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Purpose

The purpose of this study was to evaluate the effectiveness of Sterilox on controlling microbial contamination, removing biofilm and reducing endotoxins in DUWLs using the filter membrane technique, electron scanning microscopy (SEM) and kinetic chromogenic analysis (KCA).

Materials and Methods

A total of 15 dental units were selected for the study, with ten in the experimental and five in the control groups. Arrowhead distilled water (Arrowhead Mountain Spring Water Co., CA) was used as source water.

Sterilox generator (model # 9000-0010) and proprietary electrolyte solution (Lot # 070104LP2 001, expiration date 12/05) were provided by the study sponsor (Ultradent Products, South Jordan, Utah). The treatment for the experimental group followed manufacturer's instructions. After eight hours of initial shock treatment each DUWL was flushed for 30 to 40 seconds with fresh Sterilox solution. For daily usage, 15 mL fresh Sterilox solution was added to a 750 mL bottle before filling with distilled water each time.

The controls maintained current disinfection protocol of Loma Linda University School of Dentistry since 1999, i.e., DUWLs were treated with Mint-A-Kleen (Bio2000, Anodia Systems, Danville, KY) once a week; distilled water was used as source water.

Water samples collected from air/water syringes, high-speed handpieces, source water and water bottles were evaluated. The assignment of dental units to groups was determined by the baseline colony forming unit (CFU/mL). The testing schedule was as follows:

Group	N ^a	Treatment	Testing Schedule
Control	5	Bio 2000 (once/week) ^b	Baseline, 1-day & 1, 2, 4, 8-week
Experimental	10	Sterilox (20 mL/liter)	Baseline, 1-day & 1, 2, 4, 8-week

^a N: number of dental units

^b The DUWLs disinfection protocol of Loma Linda University School of Dentistry since 1999.

All DUWLs were flushed for one minute prior to sampling. Samples were collected in sterile containers and processed within one hour.

The R2A agar, petri dishes, membrane filters and sterilized test tubes were purchased from Fisher Scientific (Pittsburgh, PA). Tryptic Soy Broth (TSB) and agar were from Becton Dickson and Co. (Sparks, MD). Glutaraldehyde, alcohol and hexamethyldisilazane were obtained from Sigma Co. Cacodylate, osmium tetroxide, colloidal silver paint and all supplies for SEM were purchased from Ted Pella Inc. (Redding, CA)

Effects of Sterilox on DUWLs were evaluated by determining the CFU/mL at 22°C and 37°C before and after the treatment. Membrane filter technique was used to determine the CFU/mL. Each sample was diluted and filtered through a membrane filter of 22 µm pore size. The filter was removed aseptically and then placed on a sterile R2A agar plate. The plates were incubated at 22°C for seven days. The same water sample was also processed using the same procedures, placed on TSB agar plates and incubated at 37°C for 48 hours. Plates with 30 to 300 colonies were counted under a stereomicroscope to obtain CFU/mL values.

The biofilms of air/water syringe tubing were examined under the SEM. Tubing samples were collected at the baseline, after the shock treatment and at eight weeks. Samples were fixed in 2% glutaraldehyde in cacodylate buffer (0.1 M, pH 7.2), rinsed with cacodylate buffer, post-fixed with osmium tetroxide, dehydrated with an ascending series of alcohol solutions, and treated with HMDS (hexamethyldisilazane). The processed samples were stored in a desiccator for three days, attached to aluminum stubs with colloidal silver paint, and sputter coated with gold-palladium to approximately 15 nm thick for the evaluation using an SEM (XL30-FEG, Philips, Netherlands) at an accelerating voltage of 5 kV. Each specimen was examined at magnifications of 200x, 2,000x and 4,000x for biofilm morphology. Representative photographs were taken from each group for documentary and illustrative purposes.

One-third of samples were randomly selected to determine endotoxin levels (EU/mL) by Charles River Laboratories (Charleston, SC) using the kinetic chromogenic method, which utilizes a sophisticated kinetic spectrophotometer to monitor the color formation. A linear

relationship exists between the log of endotoxin concentration and log of reaction time. Reaction time is the time in seconds required for the standard/sample to change by a specified optical density (OD).

Means and standard deviations of CFU/mL from both 22°C and 37°C and endotoxin levels were calculated. The between-treatment effect was analyzed using the Student *t*-test, while the within-treatment effect was determined using the One-Way Analysis of Variances (ANOVA) and Student-Newman-Keuls multiple comparison method. The SEM evaluation of the biofilm morphology is descriptive.

Results and Discussion

The results of CFU/mL from 22°C and 37°C are presented in Table 1 through Table 4. The baseline CFU revealed high microbial counts in all DUWLs. The R2A plates showed an average of 111,040 and 40,600 CFU/mL in Air/water syringe lines and high-speed handpieces lines, respectively. There were no statistical differences in the baseline CFU data between the two groups. After the treatment and throughout the study the Sterilox practically eliminated the CFU in all waterlines, while the control group remained at the high CFU counts similar to baseline values; the differences between the two groups were statistically different ($P < 0.05$). Mint-A-Kleen (Bio2000) contains 0.12% of Chrohexidine gluconate, is a well-known antimicrobial agent of DUWLs. However, after six years continuing use of Mint-A-Kleen (Bio2000) as disinfectant, some microorganisms may have developed resistance to it, causing the high CFU in the DUWLs.

Figure 1 illustrates the endotoxin levels in the DUWLs at various time points. The average EU/mL changed from 0.465 at the baseline to 6.716 one day after the Sterilox shock treatment, indicating lysis of gram-negative bacteria in DUWLs. This phenomenal was observed from our previous studies. EU levels then continued to decline and were kept at an acceptable level for the rest of the study. The endotoxin in controls fluctuated in higher levels. Endotoxin is part of the outer membrane of the gram-negative bacteria, and it is capable of causing various nonspecific pathophysiological reactions. It is also a known pyrogen because of its fever induction properties. The shock treatment with high concentrations of antimicrobial agents to eliminate microorganisms in DUWLs can increase the endotoxins release within the first few days.

Figures 2, 3 and 4 are representative SEM images of biofilm. Figure 2 is the SEM image of dental tubing lumen surface before the treatment. Cocci and rods are visible on a dense, mature biofilm matrix. Figure 3 and 4 are the SEM Images of post-treatment with Sterilox. They show that the Sterilox treatment has disrupted the biofilm considerably, which has lost its typical architecture.

Conclusion

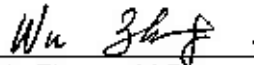
The Ultradent Sterilox is effective in controlling microbial contamination, reducing biofilm and maintain endotoxin at an accept level in Dental Unit Waterlines (DUWLs).

Good Laboratory Practice (GLP)

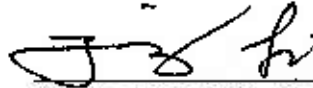
This study will be conducted following the FDA guidelines for Good Laboratory Practice for Nonclinical Laboratory Studies (21 CFR Part 58).

Reference

1. Guidelines for Infection Control in Dental Health-Care Settings—2003, Center for Disease Control and Prevention (CDC), MMWR, December 19, 2003; 52: RR-17.
2. Microbiological Evaluation of a Range of Disinfectant Products to Control Mixed-Species Biofilm Contamination in a Laboratory Model of a Dental Unit Water System; JT Walker Applied and Environmental Microbiology, June 2003; 69 (6) 3327-3332.



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Table 1. Comparison of CFU/mL in water samples collected from air/water syringes between Sterilox and control groups -- (Incubation at 22°C)

Treatment	Control (N=5)		Sterilox (N=10)		<i>p</i> -value
	Mean	SD	Mean	SD	
Baseline	111,386 ±	219,717	111,040.0 ±	51,408.0	0.996
Post-treatment	17,920 ±	4,330	0.5 ±	0.9	<0.001
1-week	16,254 ±	6,593	0.9 ±	0.9	<0.001
2-week	17,640 ±	18,151	0.6 ±	0.5	0.007
4-week	10,404 ±	7,197	4.1 ±	9.5	<0.001
8-week	8,796 ±	8,859	0.6 ±	0.8	0.006

^a Values within brackets are not significantly different as determined using the Student-Newman-Keuls method.

Table 2. Comparison of CFU/mL in water samples collected from high-speed handpieces between Sterilox and control groups -- (Incubation at 22°C)

Treatment	Control (N=5)		Sterilox (N=10)		<i>p</i> -value
	Mean	SD	Mean	SD	
Baseline	38,640 ±	30,090	40,600.0 ±	32,919.0	0.913
Post-treatment	17,440 ±	5,205	0.2 ±	0.4	<0.001
1-week	30,958 ±	22,761	0.6 ±	0.8	<0.001
2-week	83,560 ±	139,882	3.0 ±	6.2	0.071
4-week	11,337 ±	9,255	0.9 ±	0.9	0.001
8-week	10,400 ±	10,718	1.0 ±	0.8	0.007

^a Values within brackets are not significantly different as determined using the Student-Newman-Keuls method.

Table 3. Comparison of CFU/mL in water samples collected from air/water syringes between Sterilox and control groups – (Incubation at 37°C)

Treatment	Control (N=5)		Sterilox (N=10)		<i>p-value</i>
	Mean	SD	Mean	SD	
Baseline	62,594 ±	122,184	39,213.0 ±	49,759	0.600
Post-treatment	10,518 ±	8,917	0.1 ±	0.3	0.002
1-week	9,880 ±	6,689	0.4 ±	1.0	<0.001
2-week	6,008 ±	7,715	0.9 ±	0.7	0.024
4-week	12,872 ±	11,728	0.6 ±	1.0	0.003
8-week	15,946 ±	15,126	0.5 ±	0.9	0.004

^a Values within brackets are not significantly different as determined using the Student-Newman-Keuls method.

Table 4. Comparison of CFU/mL in water samples collected from high-speed handpieces between Sterilox and control groups – (incubation at 37°C)

Treatment	Control (N=5)		Sterilox (N=10)		<i>p-value</i>
	Mean	SD	Mean	SD	
Baseline	4,862 ±	4,869	22,124.0 ±	20,711	0.913
Post-treatment	12,368 ±	8,667	0.2.0 ±	0.4	<0.001
1-week	10,697 ±	7,384	0.2.0 ±	0.4	<0.001
2-week	4,640 ±	4,095	0.2.0 ±	0.9	0.003
4-week	17,656 ±	12,789	0.5.0 ±	0.5	<0.001
8-week	17,146 ±	17,034	0.3.0 ±	0.7	0.006

^a Values within brackets are not significantly different as determined using the Student-Newman-Keuls method.

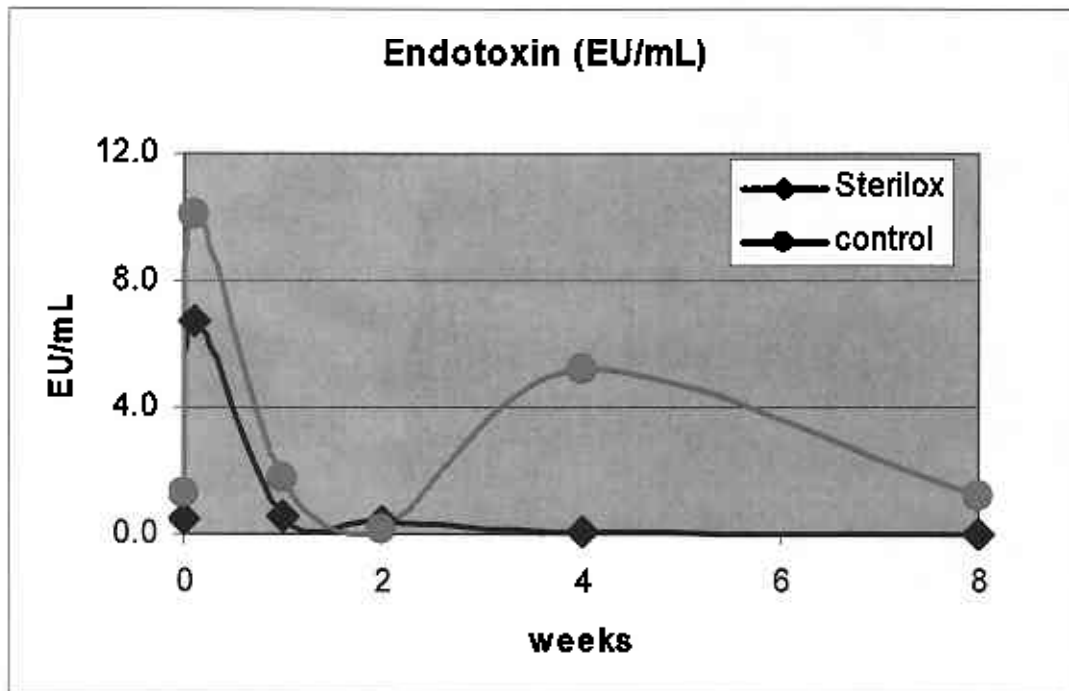


Figure 1. Comparison of endotoxin levels (EU/mL) in DUWLs samples between Sterilox and control groups

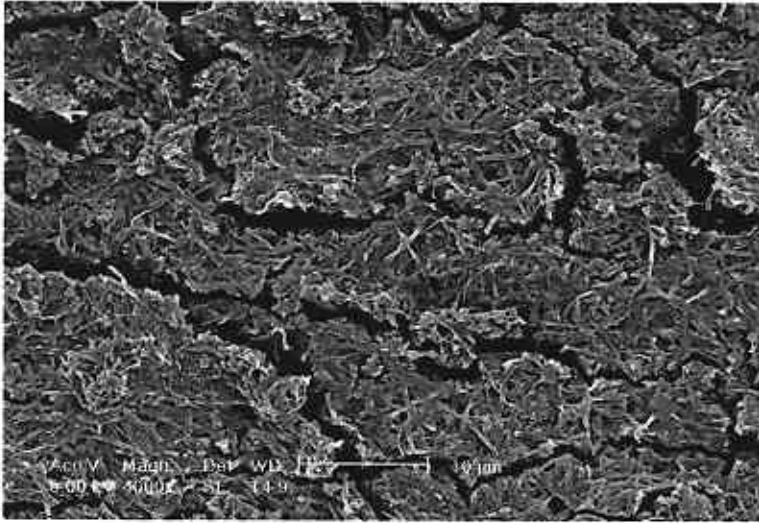


Figure 2. Air/water syringe tubing: dense, mature biofilm matrix before Sterilox treatment.

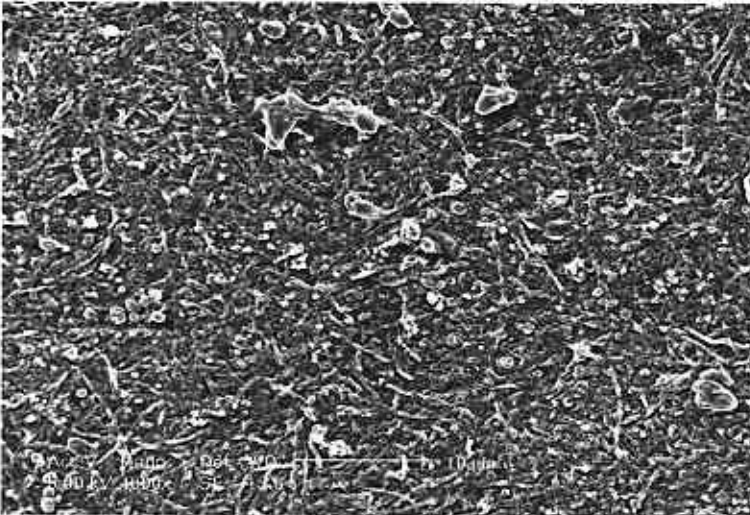


Figure 3. After Sterilox shock treatment.

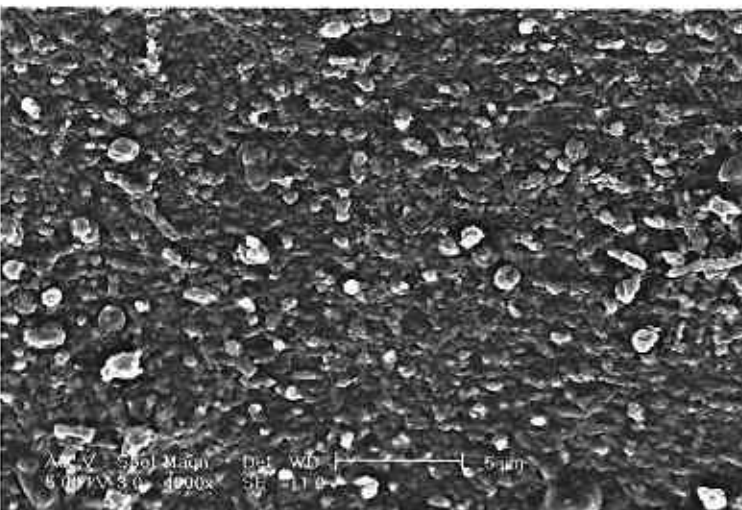


Figure 4. Eight-week treated with Sterilox.