



Controlling Bacteria Opportunistic Pathogen Contamination in DUWLs using Citrisil

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Abstract

Purpose: The purpose of this study was to evaluate the efficacy of Citrisil tablets in controlling opportunistic pathogenic microbial contamination in dental tubing, preventing biofilm formation, and potential biofilm removal from contaminated dental lines using the standard dental unit waterline biocide testing model. Materials and Methods: Regular polyurethane dental tubing (Freeman Manufacture Inc. Newberg, OR) was used. Test materials were: Citrisil daily tablets (CDT), Citrisil Enhanced Cleaner (CEC), Aseptisil-liquid and Aseptisil-powder (Sterisil, Inc. Palmer Lake, CO). Sterilex (Sterilex Co. Owings Mills, MD) served as the control during the shock treatment. A two-species inoculum, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, was used to inoculate the dental tubing for 8 hours per day. Experiments were conducted using a standard dental unit waterline biocide test apparatus and methodology as proposed by the ADA Standards Committee on Dental Products. The study duration was four-weeks. Three-overnight shock treatments were performed using the tubing with accumulated biofilm over the 4 weeks period. The effect of the test materials was evaluated using effluent planktonic microbial counts (colony forming units (CFU)/mL), biofilm total viable counts (TVC), and scanning electron microscopy (SEM). Results: The CDT eradicated 1×10^5 CFU/mL from the inocula throughout the 4 weeks period. The TVC data showed statistical differences between control and Citrisil group ($p=0.003$). CEC, Aseptisil-liquid and Aseptisil-powder effectively eradicated all the planktonic cells from tubing effluent and TVC from the biofilm during three consecutive overnight shock treatments. The test results were compatible with the Sterilex, which has the ADA acceptance seal. A single 10 minutes treatment with either Aseptisil-liquid or Aseptisil-powder was able to kill all planktonic bacteria from contaminated dental tubing. Conclusion: The daily use of CDT effectively controls opportunistic pathogenic microbial contamination in dental tubing and prevents biofilm formation. The CEC, Aseptisil-liquid and Aseptisil-powder inactivate biofilm and keep the dental tubing free from opportunistic pathogenic microbial contamination.(Sponsored by Sterisil, Inc.)

Purpose

The purposes of this study was to evaluate the effects of Citrisil Tablets in controlling bacteria opportunistic pathogen contamination and preventing biofilm formation in the dental tubing using a dental unit waterline biocide testing model and biofilm inactivation procedure.

Material and Methods

A. Test materials

- Citrisil Daily Tablets (CDT)
- Citrisil Enhanced Cleaner (CEC)
- Aseptisil™-liquid
- Aseptisil™-powder
- Sterilex Ultra Powder (SUP, as control)

B. Dental tubing

- Polyurethane dental tubing (size 1/16 ID, 1/8 OD)

C. Source water

- Sterile DI water

D. Two-species inocula

- Pseudomonas aeruginosa* (ATCC 700829)
- Klebsiella pneumoniae* (ATCC 700831)

E. Test model

- Standard dental unit waterline biocide test apparatus and methodology as proposed by the ADA Standards Committee on Dental Products WG 9.48

Figure 1. Dental Unit Water Line Biocide Test Schematic

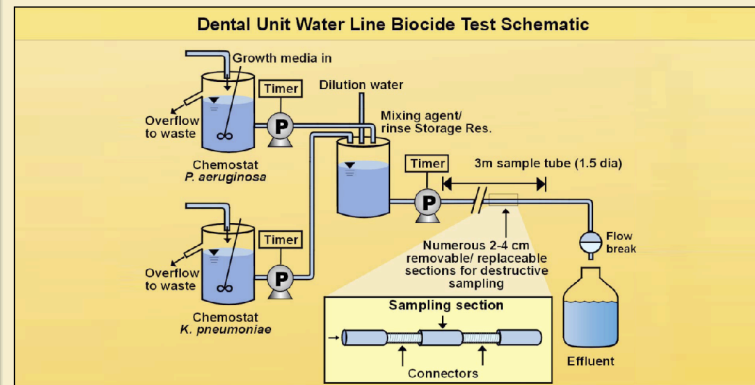


Figure 2. Dental Unit Water Line Biocide Test Model



F. Treatment regimens

Table 1. Groups and treatment regimens

Part one (4-week)	Daily treatments		
	Groups	Sterile DI water	Citrisil Daily Tablets
	Group 1	Yes	No
	Group 2	Yes	Yes
Part two (3-day)	Biofilm inactivation procedures		
		Treatments	
		Overnight	Day
	Group 3	Aseptisil™-liquid	Citrisil Daily Tablets
	Group 4	Aseptisil™-powder	
	Group 5	Sterilex powder	Sterile DI water
Part three (1-day)	Quick disinfection		
		Treatments	Contact times
	Group 6	Aseptisil™-liquid	10 min.
	Group 7	Aseptisil™-powder	10 min.
	Group 8	CEC	Overnight

G. Outcome parameters

- Microbial colony forming units (CFU/mL) of planktonic cell counts of effluent
- Biofilm total viable counts (CFU/cm²) of dental tubing
- Scanning electron microscopy (SEM) photomicrographs of biofilm

H Analyses and Interpretation of Data

Means and standard deviations of CFU/mL and CFU/cm² were calculated. The between-treatment effect was analyzed using the t-test, one-way analysis of variances (ANOVA) was performed to determine the within-treatment effect.

Results

Figure 3. Efficacy of Citrisil daily treatment in controlling planktonic cell counts of effluent

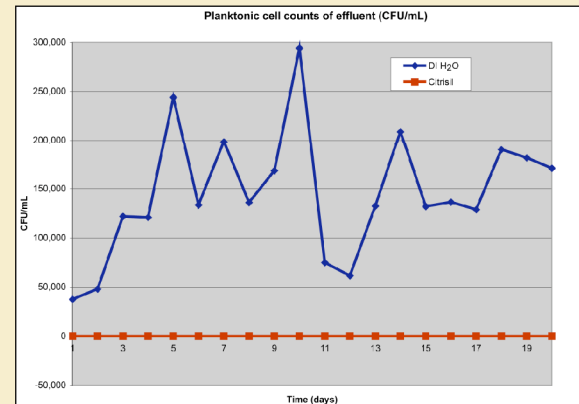


Figure 4. Biofilm accumulated in control tubing: deionized water and two-species inoculum for four weeks

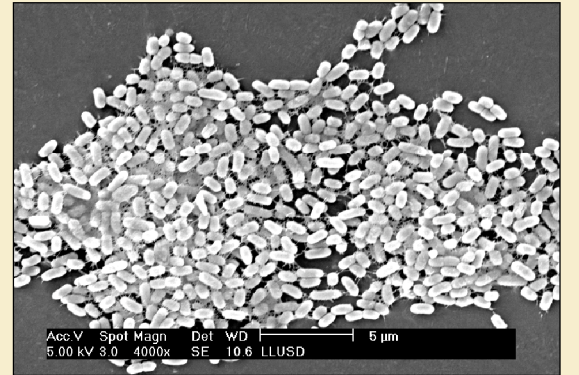


Figure 5. No biofilm formation in test tubing: Citrisil daily solution and two-species inoculum for four weeks.

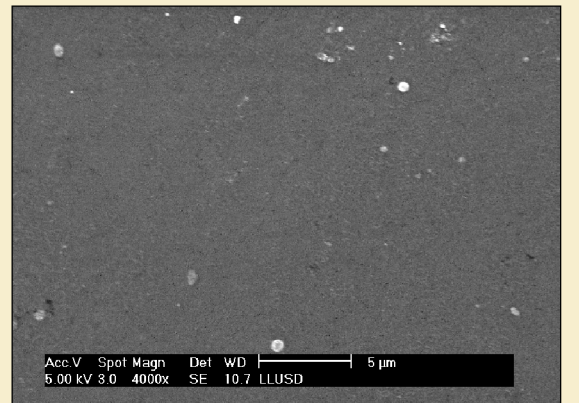


Table 2. Efficacy of Citrisil daily treatment in controlling biofilm TVC (4-week)

Groups	Treatment Two-species inocula	CFU/cm ²		t-test
		Mean	± Sd ^a	
Group 1	Sterile DI water	362,824	± 148,335	0.003
Group 2	Citrisil Daily Tablets	0.0	± 0.0	

^a N=4

Table 3. Effects of Aseptisil™-liquid, Aseptisil™-powder and Sterilex on planktonic CFU during 3-overnight treatments

Treatments		Planktonic cell counts of effluent (CFU/mL) ^a		
Days	Nights	Day-1	Day-2	Day-3
Citrisil	Aseptisil™-liquid	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Citrisil	Aseptisil™-powder	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Sterile DI water	Sterilex Powder	40.0 ± 56.6	0.0 ± 0.0	0.0 ± 0.0

^aMean ± standard deviation (N=2). Values within brackets are not significantly different as determined using the Student-Newman-Keuls method.

Table 4. Effects of Aseptisil™-liquid, Aseptisil™-powder and Sterilex on biofilm TVC during 3-overnight treatments

Treatments		Biofilm total viable counts (CFU/cm ²) ^a		
Days	Nights	Day-1 ^b	Day-2	Day-3
Citrisil	Aseptisil™-liquid	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0 ^c
Citrisil	Aseptisil™-powder	0.0 ± 0.0	0.0 ± 0.0	28.1 ± 35.8
Sterile DI water	Sterilex Powder	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

^a The dental tubing was contaminated with 3.6×10^5 CFU/cm² before the treatment;

^b Mean ± standard deviation (N=4). Values within brackets are not significantly different as determined using the Student-Newman-Keuls method;

^c No significantly different between Day-3 Aseptisil™-liquid and Sterilex-powder.

Table 5. Effects of Aseptisil™-liquid, Aseptisil™-powder and CEC on planktonic CFU

Groups	Treatment	Contact time	CFU/mL ^a
Group 6	Aseptisil™-liquid	10 minutes	0.0 ± 0.0 ^b
Group 7	Aseptisil™-powder	10 minutes	0.0 ± 0.0
Group 8	CEC	Overnight	0.0 ± 0.0

^a The dental tubing had 1.6×10^5 CFU/mL before the treatment

^b Mean ± standard deviation (N=4). Values within brackets are not significantly different as determined using the Student-Newman-Keuls method.

Conclusions

Under the conditions of the present study, Citrisil Daily Tablets are effective in controlling opportunistic pathogenic microbial contamination in dental tubing and preventing biofilm formation during the 4-week study period. Overnight treatment with either Aseptisil™-liquid or Aseptisil™-powder inactivates biofilm formation and keeps the dental tubing free of the two-species opportunistic pathogenic microbes. A single 10-minute treatment with Aseptisil™-liquid or Aseptisil™-powder eliminates the planktonic microbes in the dental tubing.