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The effect of disinfectant agents in eliminating the contamination of dental unit water

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SUMMARY High concentrations of water-borne organisms cause multiple public health problems. Contamination of water exiting the dental unit water lines could be inhibited with the use of some disinfectants. The purpose of this investigation was to establish the effect of two disinfectants and to test their capacity to eliminate colony forming units (CFU) per mL. Vacuum lines of four chairs were treated for a total of 2 weeks. Two disinfectants (Bio[®] 2000 and Alpron[®]) were used as per manufacturer's instructions. Water samples for heterothrophic counts from each unit's air/water syringe line were collected before treating the first patient of the day. Baseline, daily, first and second week samples of 10 mL were plated on blood agar plates and eosin ethylene blue agar. For mesophilic bacterial counts, Mueller Hinton agar plates with 1 mL direct and

1/10 were used in sterile serum and CFU were counted. The suspected colonies were further evaluated using API 20E and API 20NE. No Gram(-) opportunistic pathogens were found during the entire observation. Baseline contamination level (>102 CFU mL⁻¹) without use of disinfectants was significantly higher ($P < 0.0001$) than at both first and second weeks when disinfectants were used. No colony was formed when Bio 2000 was used after both first and second weeks, whereas small number of CFU mL⁻¹ was found at the end of the first week when Alpron (<10) was used. In conclusion, when used daily, both disinfectants prevent the development of bacterial contamination after first and second weeks with no significant differences ($P = 0.35$).

KEYWORDS: dental unit water, disinfectants, biofilm

Introduction

Contamination of water exiting the dental unit water lines (DUWL) with bacteria emanating from biofilm on the tubing walls has been well demonstrated (Blake, 1963; Mayo, Oertling & Andrieu, 1990; Beierle, 1993; Williams *et al.*, 1993; Atlas, Williams & Huntington, 1995). Water at the tubing walls is virtually stagnant, allowing bacteria to adhere and colonize the internal surfaces which provides a particularly favourable environment for biofilm formation. Although the biofilm remains fixed to the tubing walls, organisms often slough off into the flowing water, where they may be carried into the patients' mouth or into ambient air via spray or spatter from dental instruments. It is established that microbial contamination of DUWL is

often 100 times greater than the ADA recommended level of 200 colony forming units (CFU) per mL (ADA, 1996; Shearer, 1996).

Although the data linking poor water quality with adverse health effects in dentistry are limited (Martin, 1987), a great number of scientific evidence exists to document water-borne infections in hospital settings (Williams *et al.*, 1982; Ruf *et al.*, 1988; Alary & Joly, 1992; Karpay *et al.*, 1999; Meiller *et al.*, 1999). Contaminated water may also be ingested by the patient, contact open wounds or be aerosolized and inhaled by the patient or provider. The use of instruments such as an ultrasonic scaler, which potentially could force microorganisms into the gingiva, may raise the possibility of introducing microorganisms into the bloodstream (Blake, 1963; Reinhardt, Bolton & Hlava,

1982). Commonly used systems for preventing the contamination of water are independent water systems, chemical treatment protocols (whether intermittent or continuous), the use of filters and sterile water delivery systems (Pankhurst, Johnson & Woods, 1998).

A variety of chemical treatments (ozone, sodium hypochlorite, hydrogen peroxide, chlorhexidine gluconate, ethanol, povidone iodine) have been studied for their ability to combat biofilm and/or control dental treatment water quality. Depending on the nature of the germicidal agent, such solutions for the control of dental water contamination may be intermittently used or continuously introduced to treatment water but the consequences of chemical exposure on materials used in the construction of the unit are not always predictable (Pankhurst *et al.*, 1998).

The safety and efficacy of intermittent chemical treatment with sodium hypochlorite (1:10) is supported by the scientific literature (Abel *et al.*, 1971; Fiehn & Henriksen, 1988; Williams, Quinby & Romberg, 1994), however, this application is also found to cause some damage to the waterlines and the practitioners are warned with the material compatibility of these regimens.

Both ADA and Centers for Disease Control and Prevention (1994) guidelines for infection control in dentistry recommend flushing waterlines for several minutes before the first patient of the day is treated to remove suspended bacteria, and for 20–30 s between patients to remove material that may be retracted during treatment. Although flushing can reduce the numbers of bacteria in dental treatment water, the effects are transient. Flushing, however, was found to provide only temporary reductions in bacterial load and has no effect on the biofilm (Whitehouse *et al.*, 1991; Williams *et al.*, 1994). In most studies, reduction of the bacterial load to the desired standard of < 200 CFU mL⁻¹ was not consistently achieved, unless extended flush times were employed. Flushing for 20 min, which would be impractical in a busy dental surgery, will reduce the bacterial count to zero.

Sterile water delivery systems, on the other hand, are principally used for surgical applications, including placement of dental implants. Nonetheless, these systems are expensive to purchase and operate and often are less convenient to use than conventional delivery systems (Shearer, 1996).

The purpose of this investigation was to establish the effect of two disinfectants and to test their capacity to

eliminate CFU mL⁻¹ at baseline, after first and second weeks.

Materials and methods

Vacuum lines of four brand new, busy dental operatories at the Department of Prosthodontics at Marmara University in Istanbul were purged daily with Bio 2000* and Alpron[†] for 2 weeks as per manufacturer's instructions. Alpron includes 3% p-hydroxybenzoic acid ester, $< 0.5\%$ polyaminophylbiguanid, 15–30% 1,2-prophynglycol and Bio 2000 includes 0.12% chlorhexidine gluconate, 12% ethanol.

The waterlines were treated overnight on a daily basis at the end of each clinical day by introducing disinfectants into the waterlines. After treatment, lines were flushed with tap water for 2 min to remove residual disinfectant from the lines.

Water samples of 100 mL for heterothrophic counts from each unit's air/water syringe line were collected in separate sterile containers using aseptic techniques and labelled before treating the first patient of the day and quantified for total mean CFU mL⁻¹. The sampling was performed before work started in the morning so that the water collected had stagnated in the waterlines for 12 h. Dentists then treated patients as they normally would and subsequent samples were taken daily and tested for an average of 2 weeks. Baseline, daily samples of 100 mL for the first week and the second samples for the week thereafter were plated on blood agar plates and eosin ethylene blue agar (R2A agar at 25 °C). For meosifilic bacterial counts, Mueller Hinton agar plates[‡] with 1 mL direct and 1/10 were used in sterile serum. The suspected colonies were further evaluated using API 20E and API 20NE.

Repeated measures of ANOVA and one factor ANOVA were used to assess statistical differences between disinfectants and their effect in time differences.

Results

The mean CFU mL⁻¹ found for control and both disinfectants at baseline, first and second weeks are presented in Fig. 1 and Table 1.

*Bio 2000, Micrylium Laboratories, Toronto, Canada.

[†]Alpron, Alpro[®] Dental, Schwarzwald, Germany.

[‡]Dalynn Biologica LB, Alberta, Canada.

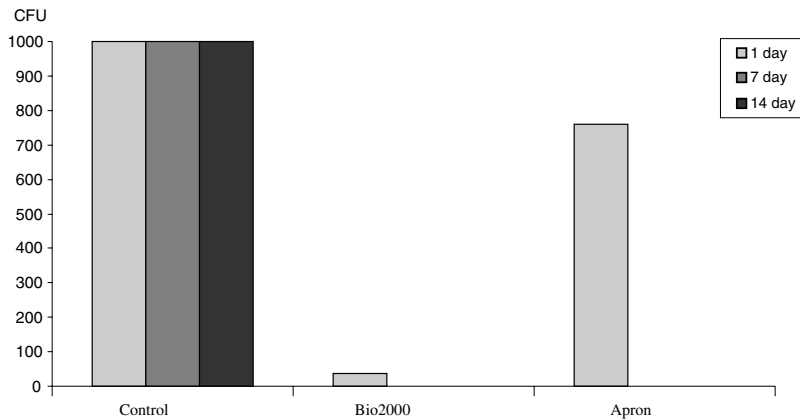


Fig. 1. Mean values of CFU mL⁻¹ at baseline, 1 and 2 weeks after the DUWL were purged with disinfectants in comparison with the control group.

Table 1. Mean values (CFU mL⁻¹) and standard deviations at baseline, 1 and 2 weeks after the DUWL were purged with disinfectants

	Bio 2000	Alpron
Baseline	36.25 (57.06)	760 (480)
First week	0 (0)	0.75 (1.5)
Second week	0 (0)	0 (0)

Results of two factors repeated measures ANOVA showed significant differences between disinfectants and the control group ($P = 0.024$) (Table 2).

Baseline contamination level, which is the first day of the application, expressed as the mean CFU mL⁻¹ without use of disinfectants ($>10^2$ CFU mL⁻¹) was found to be significantly higher ($P < 0.0001$) than those obtained with the treatment of the lines with Bio 2000. There was no statistically significant difference between control and Alpron which is the first day application ($P = 0.35$).

There was a significant difference between Bio 2000 and Alpron ($P < 0.024$) on the first day of application. The mean CFU mL⁻¹ between the baseline in the control group and at the second week, when disinfectants were used (Alpron:0 and Bio 2000:0), were significantly different ($P < 0.0001$).

No colony was formed when Bio 2000 was applied after the first week, whereas small number of CFU mL⁻¹ was found at the end of the same duration when Alpron (< 10) was used.

Both disinfectants prevent the development of bacterial contamination after the second week as bacterial counts showed 0 CFU mL⁻¹.

Further microbiological evaluations demonstrated no Gram (-) opportunistic pathogens during the entire observation either with or without the use of disinfectants.

Discussion

The presence of adherent microbial biofilms in dental waterlines has been described for many years (Blake, 1963). The interest in these biofilms has been reawakened recently due to increasing number of immunocompromised dental patients and also due to an increase in awareness of occupational hazards in the dental offices (Costerton, Lewandowski & Caldwell, 1995). In spite of absence of any definable health effects associated with dental unit waters, the ADA recommended maximum permissible levels of < 200 CFU mL⁻¹ (ADA, 1996).

In recent years, a number of means for the control of microbial colonization have been suggested or evaluated.

Source	d.f.	Sum of squares	Mean square	F-test	P-value
Disinfectants	1	262450.125	262450.125	9.041	0.0238
Subjects w. Groups	6	174163.875	29027.312		
Repeated measure (B)	3	5356424.75	178547.917	61.006	0.0001
AB	3	785179.125	261726.375	8.943	0.0008
Bx subjects w. groups	10	526811.625	29267.312		

Table 2. ANOVA Table for 2-factor repeated measures

There has been significant progress in developing reliable, economical methods to prevent or control the formation of microbial accumulations in DUWL. Various publications have recommended different biocides (compounds with lethal activity against living organisms) for use in dentistry including sodium hypochlorite, chlorhexidine gluconate, povidone iodine, ethanol, peroxide and glutaraldehyde (Miller, 1994; Williams, Andrews & Santiago, 1996; Pankhurst *et al.*, 1998). Filtration, disinfection with biocides, UV light, ozone and independent sterile water reservoirs or combination of methods have all been advocated to control DUWL contamination.

Biocides are employed to remove the biofilm and eliminate the planktonic bacterial count. The results show that the water going into the dental units was more frequently contaminated in the dental school (53%) (Zanetti *et al.*, 2000). Considering the drawbacks of other methods than biocides, the objective of this study was to investigate the effect of routine use of two disinfectants and to test their capacity to eliminate CFU mL⁻¹ in the dental school.

In this study, the baseline contamination level expressed as the mean CFU mL⁻¹ without use of disinfectants (> 10² CFU mL⁻¹) was found to be higher than the recommended level by ADA (< 200 CFU mL⁻¹). However, this value is also less than other reported baseline values in similar studies (Kettering *et al.*, 1997; Puttajah *et al.*, 1999, 2001). One explanation for this could be that the dental operatories used in our study were brand new.

Immediately after the use of the disinfectant, a dramatic decrease was observed with the use of Bio 2000 than that of Alpron. This might be due to the chlorhexidine gluconate content of the Bio 2000 which requires further evaluation.

To date there is no published evidence of a serious public health risk from biofilm-contaminated dental unit water. Most of the microorganisms found in dental water are Gram (-), heterotrophic bacteria that have little potential to cause disease in immunocompetent people (Zanetti *et al.*, 2000). One study used non-sterile and sterile water to examine the incidence of bacteraemia after ultrasonic root scaling (Reinhardt *et al.*, 1982). Although investigators found no significant difference between the two methods in the number of bacteria entering the bloodstream, higher numbers of Gram (-) bacteria were noted in the group treated with non-sterile water. It was emphasized that the presence

of high levels of opportunistic organisms may overload the defence systems of immunocompromised patients and occupationally exposed dental staff members. In this study, microbiological evaluations demonstrated no Gram (-) opportunistic pathogens during the entire observation either with or without the use of disinfectants.

Clinically important opportunistic Gram (-) pathogens are *Pseudomonas* spp., *Klebsiella* spp. as well as fungi, free living amoebae, protozoa and nematodes. Only *Pseudomonas aeruginosa* derived from DUWL has definitely been shown to cause oral infection in patients (Zanetti *et al.*, 2000). Although no attempt was made to identify the species of microorganisms, currently existing disinfectants should be evaluated for their effect on different pathogens.

Once a new DUWL system is connected to the main water supply, even when it is not used for patient treatment, a biofilm will form in 8 h (Tall *et al.*, 1995). Currently there is no clinical evidence of a widespread public health problem from exposure to DUWL. Nevertheless, removal of these substances from water delivered into patients' mouths may reduce the potential for post-treatment inflammatory episodes.

The results of this study, in compliance with others (Mills, Lauerdale & Mathew, 1986; Fiehn & Henriksen, 1988; Douglas & van Noort, 1993; Williams *et al.*, 1996), proved the benefit of disinfectants for eliminating the CFU in routine use. By the end of the second week, both agents exhibited no CFU. The duration of their effectiveness needs to be studied.

As the benefits of the disinfectants for DUWL are obvious, the use of disinfectants should be encouraged in dental schools or hospitals.

Conclusions

1. Baseline contamination level expressed as the mean CFU mL⁻¹ without use of disinfectants (>10² CFU mL⁻¹) was found to be significantly higher ($P < 0.0001$) than those obtained at baseline with the treatment of the lines using Bio 2000.
2. Both Alpron and Bio 2000 appear to be an effective disinfectant for use in eliminating the CFU in DUWL totally at the end of 2 weeks.
3. Microbiological evaluations demonstrated no Gram (-) opportunistic pathogens during the entire observation either with or without the use of disinfectants.

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