

Important Results Lead a Guideline on the Use of Ozone for Water and Dental Equipment Disinfection: An Original Study

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Abstract

Studies have shown that the ozonated water can be utilized in the reducing infections caused by oral microorganisms and in the biological control of water units of the dental equipment. The aim of this study was to evaluate the disinfectant activity of ozonated water on equipments and dental countertops by using equipment manufactured by domestic company (Bottle-Q2 TEC, Barretos, SP). To do so, 6 equipments were selected randomly, the Integrated Clinic of the University Center of the Educational Foundation from Barretos, UNIFEB, SP, and they were subjected to surface disinfection tests. Samples of the operating table (MO), the triple syringe (ST) and spittoon (CUS) were obtained before (T0) and 5 minutes (T5) after the disinfection of surfaces with ozonated water (test group). The water used for the disinfection of surfaces had final concentration of 1.8 ppm ozonated water. The control group was consisted from routinely carried out disinfection in the dental clinic UNIFEB. The microbiological testing was performed by identification and counting of colony forming units (CFU/mL) by seeding each sample plaque with specific culture media for microorganisms: *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), total coliforms, fungi and yeasts. The plates were incubated at 37°C for 48 hours in bacterial greenhouse. The results showed that the ozonated water generated by Q2Tec equipment (test group) for the disinfection of dental equipment surfaces was able to reduce the surface microbial colonization of the dental equipment after 5 minutes of action ($p \leq 0.01$, ANOVA-Tukey). It is concluded that ozonated water may be an option in controlling contamination during cleaning the dental clinic.

Key Words: Ozone, Antimicrobial activity, Dental units, Biosecurity

Introduction

One of the major challenges facing dentistry today is to halt the spread of infections in clinics, which have been intensified by the increased resistance of microorganisms, the lack of care of some professionals and the high risk of transmission of infectious and contagious diseases [1-3].

Dentistry is a profession characterized by the exposure of both the professional and his or her staff to a variety of infectious agents, making them susceptible to more than 200 different diseases that can be transmitted from exposure to blood. Dental office groups a site of continuous infection, which may be direct or crossed [4,5]. Additionally, great emphasis has been given in the use of high-rotation, due to its cooling operation lead to the production of aerosols with high potential contamination of the dental environment [6].

The use of high-speed turbines, ultrasonic scrapers and air/water syringes causes aerosol to form. By aerosol is meant any volume of air containing solid or liquid particles in suspension. These particles can remain floating for a short or long period, depending on their size [7,8].

Since dental work involves the production of aerosols capable of reaching distance of 1.5 to 2 meters. All persons involved in a dental care, as well as all dental equipment located in this area are subject to contamination by bacteria, viruses and fungi that can cause various diseases [7-10].

For performing surface disinfection, various chemical disinfectants may be used [11]. The initial step for the disinfection process is the knowledge of each of these products, in its main aspects as: its mechanism of action on microorganisms, toxicity to the manipulator and deleterious action for the equipment to be disinfected. The proper choice of disinfectant provides the success of the disinfection process [12].

The disinfectants used in the course of dentistry of UNIFEB are sodium hypochlorite 1.0% and alcohol 70.0%. Indicated for disinfection of semi-critical instruments, surfaces, molds, clothes and water 1.0% sodium hypochlorite has its advantages as a fast, broad spectrum, economical, effective antimicrobial action in dilute solutions. The disadvantages include: being sporadic in high concentrations (5.25%), cannot be reused, should be prepared daily, decreased activity in the presence of organic matter, unpleasant odor, irritating to skin and eyes, corrosion of metals, damage of plastic and rubbers.

Disinfection of the surfaces is done using the chemical agent containing the detergent; the surface must be cleaned with the product to remove dirt, using mechanical action and applies the product again, leaving it in contact with the surface for 10 minutes [12]. When using a non-detergent chemical agent, wipe the surface with soap and water or detergent, remove dirt, rinse thoroughly to eliminate soap and detergent residues, and disinfect the cleaner with the surface for 10 minutes. When disinfecting contaminated areas, localized

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contamination (with presence of blood, excretions or secretions), rubber gloves should be applied, sodium hypochlorite applied at 1.0%, leave to act for 10 minutes, remove with paper towel or cloth clean, despise, and clean with soap and water [12-14].

Among the various disinfection techniques of the dental equipment tested, some studies have indicated the use of ozone gas (O₃), which has demonstrated a higher oxidizing potential than chlorine in the control of cross infection and microbiological control in clinical and surgical environments in the area of Cheers. Ozone has been used in the food industry, in the treatment of water for reuse and in effluents, and in the health of people [15].

Nowadays, oxygen and ozone have been added, due to their bactericidal properties, forming the ozonated water, which has the following advantages: being simple and inexpensive to operate, ozone leaves no residue, and converts to oxygen in a short space of time, and does not affect titanium, chromium and silicon [16]. The use of ozonated water in order to minimize the presence of contamination in water lines was employed in different studies, which demonstrated an efficient disinfecting action of ozone. Thus, to evaluate the disinfecting effect of ozonated water on the surface decontamination of dental equipment is of paramount importance for the control of cross-infection in dental practice [17].

The objective of the present study was to evaluate the antimicrobial activity of ozonated water on dental equipment and benches through the use of equipment manufactured by a national company (Garrafa-Q2 TEC, Barretos and SP).

Materials and Methods

The pilot study was divided into two groups, one being the Control Group and the other Test Group. The Control Group is represented by the research on the disinfectant action of the products recommended and used by the Dental Clinic of the University Center of the Educational Foundation of Barretos. The Test Group consists of the research on the disinfecting action of ozonated water.

The experimental design consisted of the collection of swabs, the surface of the operative table (MO), the triple syringe (ST) and the cuspeira (CUS). The samples were obtained before (T0) and 05 minutes (T5) after the cleaning of the equipment with the use of chlorine disinfectant used in the

disinfection routine of the Dental Center of the University Center of the Educational Foundation of Barretos (Control Group). Subsequently, following the same methodology, samples of clean equipment with ozonated water produced by the Garrafa Q2- TEC, (Barretos-SP-Brazil) (Test Group) were collected. They were submitted to microbiological analysis.

An ozone generator, manufactured by a national company (Q2 TEC, Barretos, SP) was used during the disinfection and asepsis of six randomly selected dental teams, at the Integrated Clinic of the University Center of the Barretos Educational Foundation, UNIFEB, SP.

The equipment was used to carry out the disinfection of the mentioned places. In order to do so, 500.0 mL of distilled water was used in the equipment reservoir which, after activation, was able to generate ozonated water with a final concentration of 1.8 ppm of ozone.

The microbiological analysis was done by means of identification and counting in colony forming units (CFU mL⁻¹) by sowing each sample in plates with culture medium specific for the microorganisms: *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), total coliforms, fungi and yeasts.

The collected material was packed in test tubes containing 0.5% sterile saline solution for later microbiological analysis. The samples were processed and analyzed within 2 hours after collection.

The samples immersed in sterile saline solution were subjected to vigorous agitation for one (1) minute to allow the release of the microorganisms collected in the liquid medium. Subsequently, the solutions containing the samples were submitted to serial dilution in saline solution (10-1 to 10-4). Approximately 25.0 µL of each dilution were seeded into petri dishes containing culture media specific for *E. coli* (Methylene Blue Eosin Agar-EMB), *S. aureus* (DNase Agar), Total Coliforms, Fungi and Yeast (Muller- Hinton-MIH).

The plates were incubated at 37.0°C for 48 hours in a bacteriological oven. The colonies were counted in colony forming units (UFC mL⁻¹). The results were presented in the descriptive form of reduction percentage of CFU mL⁻¹.

Results

Table 1. Microbial colonization of the surfaces (CFU mL⁻¹) in the groups and periods tested (mean ± standard deviation).

Bacteria/Culture media	Groups	Disinfection areas					
		CUS		ST		MO	
		T0	T5'	T0	T5'	T0	T5'
<i>E. coli</i> / EMB	Control	2.3 × 10 ³ (± 1.1) ^a	0.06 × 10 ³ (± 0.5) ^b	1.9 × 10 ³ (± 0.3) ^a	0.2 × 10 ³ (± 0.3) ^b	1.3 × 10 ³ (± 0.4) ^a	0.0 ^b
	Test (O ³)	2.5 × 10 ³ (± 0.8) ^a	0.0 ^b	1.8 × 10 ³ (± 0.3) ^a	0.0 ^b	1.3 × 10 ³ (± 0.3) ^a	0.05 × 10 ³ (± 0.04) ^b
<i>Staphylococcus</i> sp/ DNAase	Control	34 × 10 ³ (± 8.9) ^a	1.5 × 10 ³ (± 0.5) ^a	4.6 × 10 ³ (± 0.5) ^a	3.9 × 10 ³ (± 1.1) ^a	224 × 10 ³ (± 43.4) ^a	13.2 × 10 ³ (± 3.9) ^b

	Test (O ³)	21.2 × 10 ³ (± 12) ^a	0.02 × 10 ³ (± 0.04) ^b	2.0 × 10 ³ (± 0.6) ^a	0.0 ^b	168 × 10 ³ (± 39) ^a	10.8 × 10 ³ (± 0.7) ^b
^a different letters in the lines, mean presence of a statistically significant difference between the periods within each group and disinfection area, for p ≤ 0.01 (ANOVA-Tukey). ^b Symbols in the columns mean presence of a statistically significant difference between the groups in the disinfection areas, for p ≤ 0.01 (ANOVA-Tukey)-There was no difference.							

The ozone water generated by the Q2Tec (test group) in the disinfection of dental equipment surfaces was able to reduce the microbial colonization of the surfaces of the dental equipment after 5 minutes of action, being statistically significant ($p \leq 0.01$). The solution used in the routine disinfection of the dental clinic of UNIFEB (control) statistically reduced microbial colonization in the cuspeira and in the operative table, however, no statistical difference was observed for the triple syringe.

The disinfection methods tested did not present statistical difference in the reduction of the analyzed microorganisms.

The results can be seen in *Table 1*.

Discussion

The results demonstrated the effect of the ozonated water generated by the Q2Tec equipment in the disinfection of surfaces of dental equipment demonstrating similar effect to the solution used in the routine disinfection of the dental clinic of UNIFEB, being in agreement with studies that reported that ozone has been used as a substitute of chlorine, demonstrating efficacy in inactivation of bacteria, viruses and protozoa and in the destruction of biofilm, and can be used in decontamination of contact surfaces of equipment and in hydraulic circuits [1-5].

In view of these findings, it can be said that ozonated water is an option in the control of cross-infection odontológica [6-9], since ozone exerts a powerful germicidal effect due to its high oxidant potential of the elements that constitute the cell walls, thus penetrating into the microorganisms and oxidizing enzymes, proteins and nucleic acids, which leads to the destruction and death of the microbial cell [18-22].

It is believed that the verification of the effectiveness of ozonated water as a disinfectant agent brings additional benefits in the control of cross-infection in the dental clinic, since it is a substance that is quickly obtained and easy to apply [23-27].

The antimicrobial and solvent activities of sodium hypochlorite depend on the concentration of the chemical solution [28-32]. More concentrated sodium hypochlorite solutions have higher antimicrobial activity. Chlorinated solutions are unstable by nature and, therefore, lose the concentration of active chlorine over time [33]. In order for sodium hypochlorite solutions to be able to exert their full effectiveness, it is necessary that the concentration be the most faithful to the one indicated on the label by the manufacturer, ie the product must be of good quality [34].

The brightness and heat may interfere with the stability of the product and, therefore, it should be stored in an amber glass container. In order to avoid loss of key properties,

NaOCl solutions must be purchased within the expiration date and as close as possible to the date of manufacture [35].

The potent antimicrobial activity of NaOCl at 1.0% and 5.0% in solutions, eliminated all the bacteria surveyed at all times, using the contact test. For the diffusion test on agar, 5.0% hypochlorite was more effective on *E. faecalis*, *E. coli* and *S. aureus* [33-35].

Conclusion

The ozonated water generated by the Q2 TEC equipment was able to reduce the microbial load of dental equipment surfaces. Ozonized water may be an option in controlling contamination during cleaning of the dental clinic.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Aguiar CM, Pinheiro JT. Avaliação bacteriológica da qualidade da água utilizada nos equipos odontológicos. *Revista Da Associacao Paulista De Cirurgioes Dentistas*. 1999; **53**: 228-235.
2. Blake GC. The incidence and control of bacterial infection in dental spray reservoirs. *British Dental Journal*. 1963; **115**: 413-446.
3. Bocci V. Ossigeno ozonoterapia: Compresione dei meccanismi di ozono e passibilita terapeutiche. Milano, Ambrosiana. 2000.
4. Bocci V. Ozone, a New Medical Drug (1st edn). Dordrecht, The Netherlands. 2005.
5. Borin G, Oliveira EPM, Becker AN, Melo TAF. Utilização e forma de armazenamento da solução de hipoclorito de sódio por cirurgiões-dentistas. *Stomatos*. 2006; **12**: 25-30.
6. Clegg M. Dental unit waterlines in media spotlight. *Comuniquè*. 1996; **12**: 3.
7. Coleman DC, O'donnell MJ, Shore AC, Russell RJ. Biofilm problems in dental unit water systems and its practical control. *Journal of Applied Microbiology*. 2009; **106**: 1424-1437.
8. Costa H, Daniel L. (2002) Estudo do comportamento do ozônio na desinfecção de águas residuárias domiciliares. PROSAB edital 3, Dezembro.
9. Dhalen G, Alenas-Jarl E, Hjort G. Water quality in water lines of dental units in the public dental health service in Goteborg, Sweden. *Swedish Dental Journal*. 2009; **33**: 161-172.
10. Fantinato V, Silva MV, Almeida NQ, Jorge AOC, Shimizu MT. Exame bacteriológico da água em clínica odontológica. *Revista Da Associacao Paulista De Cirurgioes Dentistas*. 1992; **46**: 829-831.
11. Faria SI, Uerro M, Ifo CYIK, Urruchi WI, Balducci I, et al. Effects of Ozonated water on Candida albicansoral isolates. *Brazilian Journal of Oral Sciences*. 2005; **4**: 783-786.
12. Guzel-Seydim ZB, Greene AK, Seydim AC. Use of ozone in food industry. *Lebensmittel-Wissenschaft und -Technologie*. 2004; **37**: 453-460.
13. Jannuzzi H. Ozone in water treatment cooperative research report. 1991.

14. Jacinto RBS. Ozonização para controle microbiológico de linhas d'água em equipos odontológicos. Dissertação mestrado em Engenharia Biomédica. São José dos Campos. 2012.
15. Khadre JG, Yousef AE, Dave S. Application of ozone for enhancing the microbiological safety and quality of foods: A review. *Journal of Food Protection*. 1999; **62**: 1071-1087.
16. Lopes WRR, Oliveira RMS, Serra JCV. avaliação comparativa entre os métodos de desinfecção empregando cloro e ozônio de águas destinadas ao abastecimento de pequenas comunidades. *Periódico Eletrônico "Fórum Ambiental da Alta Paulista"* 2013; **9**: 463-472.
17. Mahapatra AK, Muthukumarappan K, Julson JL. Appliation of ozone, bactericides and radiation in food processing: A review. *Critical Reviews in Food Science and Nutrition*. 2005; **45**: 447-461.
18. Mills SE, Karpay RI. Dental waterline and biofilm- searching for solutions. *Compendium of Continuing Education in Dentistry*. 2002; **23**: 237-258.
19. Ministério Da Saúde. Controle de infecçõese a práticaodontológicaem tempos de AIDS. Brasília: Manual de Conduas. 2000.
20. Nicoletti MA. Estudo da estabilidade de soluções de hipoclorito de sódio Tese de Mestrado. Universidade de São Paulo, São Paulo. 1994.
21. Nikaeen M, Hatamzadeh M, Sabzevari Z, Zareh O. Microbial quality of water in dental units waterlines. *Journal of Research in Medical Sciences*. 2009; **14**: 297-300.
22. Pécora JD, Estrela C. Hipoclorito de sódio. Ciência Endodôntica, Artes Médicas, São Paulo. 2004.
23. Percival RS, Devine DA, Nattress B, Kite P, Marsh PD. Control of microbial contamination in dental unit water systems using tetra-sodyum EDTA. *Journal of Applied Microbiology*. 2009; **107**: 1081-1088.
24. Rice JO. Uses of ozone in drinking water treatment. *American Water Works Association*. 1981; **73**: 44-57.
25. Samaranayake LP, Scheutz F, Cottone JA. (1995) Controle da infecção para a equipe odontológica. São Paulo: Editora Livraria Santos.
26. Santos FS. Manual de biossegurança do curso de odontologia da fundação educacional de barretos. 2010.
27. Sassone LM, Fidel RAS, Murad CF, Fidel SR, Hirata Jr R. Antimicrobial activity of sodium hypochlorite and chlorhexidine by two different test. *Australian Endodontic Journal*. 2008; **34**: 19-24.
28. Sciaky I, Sulitzeanu A. Importance of dental units in the mechanical transfer of oral bacteria. *Journal of Dental Research*. 1962; **41**: 714.
29. Silva CRG, Jorge AOC. Evaluation of surface disinfectants utilized in dentistry. *Pesquisa Odontologica Brasileira*. 2002; **16**: 107-114.
30. Teixeira M, Santos MV. Responsabilidade no controle de infecção. *Revista Da Associacao Paulista De Cirurgioes Dentistas*. 1999; **53**: 177-189.
31. Terao R, Terao C, Mori K, Matsui M. Technical note: 100% ozone-treatment system of bath water. *Ozone-Science & Engineering*. 2003; **25**: 345-349.
32. Velano HE, Nascimento LC, Barros LM, Panzeri H. Avaliação *in vitro* da atividade antibacteriana da água ozonizada frente ao Staphylococcus aureus. *Pesquisa Odontológica Brasileira*. 2001; **15**: 18-22.
33. Watanabe E. Avaliação de níveis de contaminação da água de equipos odontológicos. Dissertação Mestrado. Faculdade de Ciências Farmacêuticas de Ribeirão Preto. 2003.
34. Williams JF, Johnston AN, Johnson B, Huntington MK, Mackenzie CD. Microbial contamination of dental unit waterlines: Prevalence, intensity and microbiological characteristics. *The Journal of the American Dental Association*. 1993; **124**: 59-65.
35. Williams HN, Johnson A, Kelley JI, Baer ML, King TS, et al. Bacterial contamination of the water supply in newly installed dental units. *Quintessence International*. 1995; **26**: 331-337.