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ORIGINAL ARTICLE

Efficacy of water filters for dental chair units: assessment of the filtration action versus Coxsackievirus B5

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Keywords

Dental chair • Hydraulic system • Biological safety • Microbiological tests • Coxsackie virus

Summary

Introduction. The microbiological safety and control of the water used in dental practice has a critical importance for avoiding crosslinked infections in the dental office. The aim of this study was to establish coxsackie virus filtration of the water applied to a dental unit. **Methods**. A specific water filter-system was used, to verify the viral load in the outgoing water. The statistical analysis was performedusing the Shapiro-Wilk and t-Student test.

Introduction

A dental unit is a system that delivers water to different points by a hydraulic system (HS): glasses for patients, water bottle tanks, ultrasonic scalers, handpieces for highspeed drills, and air and water syringes. To prevent the HS from being contaminated, the handpieces are manufactured with anti-retraction valves to prevent suck-back of contaminants from the oral cavity [1]. Water systems of domestic and old buildings, can be contaminated with legionellae and therefore represent a potential risk for the patients. Many microorganisms are detected in the hydraulic system of dental chairs such as *Ralstonia pickettii*, and *Sphingomonas paucimobilis, Pseudomonadaceae species*, including *Burkholderia cepacia, Chryseomonas luteola, Pseudomonas fluorescens* [2].

Natural and artificial water contains many microorganisms, fungi, bacteria and viruses [3, 4].

Coxsackieviruses are nonenveloped, positive-sense, single-stranded RNA viruses of the *Picornaviridae* family, genus *Enterovirus*. Enteric viruses are resistant in the environment, especially in water, and transmitted via the fecal-oral route, and by inhalation of water aerosolved particles [5].

These microorganisms can reach water systems, causing a potential risk of contamination to human health [6, 7]. In particular, they could contaminate dental units, increasing risk of cross- infection during surgery. Dental chair units contain systems providing water to cool soft and hard tissues during surgery and to cool down

Results. The outcome of the evaluation of the virologic tests shows an excellent capability of virus filtration that attested 99.9999% in the volume analyzed. A statistical difference was found in the bacterial water contamination parameter before and after filtration. (P = 0.000000).

Conclusions. According to the tests, medical devices applied to a dental unit are able to filter viruses and therefore reduce risk of contamination in the dental office.

instruments and burs used in dental procedures. The Italian Ministry of Health in 2015 issued guidelines for the prevention of *Legionella*, according to which the dentist is responsible for both the sterility of the surgical environment and of the microbiological safety of the water circulating in the dental unit.

Then there is the possibility of cross contamination by inhalation of aerosol containing pathogenic originating from spray or rotating or ultrasonic instruments if *Legionella* or other bacteria or viruses are present in the circulating water systems [8]. In addition, there is an increased risk of contamination, during surgery, caused by the direct contact of spray or water with the patient's blood [9].

In order to reduce microbial contamination and the biofilm formation in the hydraulic system in a dental unit, it is recommended to disinfect the water, and to install filters able to reduce the microbial load [10, 11]. The aim of the present study was to investigate the capacity of a medical device of ultrafiltration for dental units to filtrate Coxsackieviruses.

Materials and methods

A total 18 filters of ultrafiltration were used in this study. The water filter used has a nano-reticular structure $0.05 \,\mu$ with activated vegetable carbon added as a purifying and anti-odour agent for incoming water upstream of the dental unit, called Koala[®] filter (ODONTOKOALA

Rome, Italy). This dental unit water filter system contains the finest coconut shell based granular activated carbon. At the Department of Medical, Oral and Biotechnological Sciences of the University of Chieti-Pescara the tests were performed on the microbial colonies. The sterilizing efficacy of the filter under test was analysed towards microbial loads of Coxsackievirus B5, cultivated in monkey kidney cultures (RC37).

Two liters of sterile distilled water were contaminated with the viral culture at known titer.

Two liters of sterile distilled water were filtered to check for any initial contamination of the filtering system, then the contaminated sample was filtered (continuous physiological solution), with the same device.

The determination of the microbial load at 37° C on *Plate Count Agar* (PCA) was performed: the contaminated physiological sample was inoculated in cell cultures to verify initial viral titer. The filtered physiological sample was inoculated in cell cultures to determine residual viral load. Viral titer was established according to the Reed-Muench method, and was expressed in TCID50/25 µl [12].

Two trials were performed with Coxsackievirus B5 at initial titer of 10^6 TCID₅₀/25µl and 10^5 TCID₅₀/25µl, by using a new filter. The water for dental use is considered contaminated when waterlines count are between 10,000 and 10,000,000 cfu/ml.

STATISTICAL EVALUATION

A power analysis was performed using clinical software, freely available on the site http://clincalc.com/stats/ samplesize.aspx, for determining the number of filters needed to achieve statistical significance for quantitative analyses of quantization of virus. A calculation model was adopted for dichotomous variables (yes/no effect) by putting the effect incidence designed to caution the reasons 15% for controls and 75% for treated. Alpha was set at 0.05, Beta at 0.3 and power at 0.7. The optimal number of filters for analysis is 16 implants.

The Shapiro-Wilk test was adopted to evaluate the normality of the study data and the viral quantitative differences between before and after water filtration were analyzed by the t Student Test.

A p-value ≤ 0.05 was considered statistically significant. Statistical analysis was performed using the Statview software from SAS Institute.

Results

The filters showed an effective retention capacity of the viral loads under examination, always recording a residual zero load, even in the presence of high initial contaminant loads (10⁶ ufc/ml) with Coxsackievirus B5 used and in every case, demonstrating a reduction of 99.9999% in the volume analysed.

A statistical difference was found in the viral water contamination before and after filtration. (P = 0.000000). In the tables below are the diagrams of the results obtained (Tab. I).

Discussion

In the present study Coxsackievirus B5 was used for its small size 30nm and if the filter works against this virus it will certainly work for *Legionella* which has a much larger size. Legionellae appear to have a coconutbacillary shape with dimensions ranging from 0.3 to $0.9 \,\mu\text{m}$ wide and 1.5 to 5 μm of length.

Safety of patients and dental personnel requires the appropriate microbiological water quality in dental units [11]. During treatment, patients and dental workers are exposed both to direct contact with microbial-contaminated water in the form of splatter and contaminated aerosol emitted during work with unit handpieces, including rotating and ultrasonic instruments [3]. This is very important as it concerns medical and legal aspects.

Therefore, the use of a hygiene protocol, that guarantees a disinfection of the hydraulic system of the dental unit, is crucial in preventing cross-contamination, and to ensuring work safety, according to the current laws.

In accordance with the experiment, we can affirm that filters applied to a dental unit are able to guarantee disinfection of the water, acting as actual barriers, impermeable to the passage of microorganism.

A 0.05 μ ultrafiltration system was used, provided with a nanopore physical barrier, impermeable to viruses and bacteria, acting effectively and in safety, over a period of time equal to a year of work (8,000 liters) [11].

Therefore, the ultrafiltration system device was tested towards Coxsackievirus B5, a microorganism with equal or inferior dimensions to *Legionella*, in order to assess the absence of contamination in the water transiting in the filtering system.

The filter system was prior tested with sterile saline water to certify its sterilization in order to not null the results.

Activated vegetable carbon was placed inside the filtering system. This is, generally used to eliminate odours and chlorine from water, as well as to prevent the formation of algae in water circuits, but at the same time collects the bacterial charge beyond the membranes, for which the efficiency test performed on the filters used represents a significant data on the biological safety of the filter.

Conclusions

The results achieved showed that the filter system was able to completely eliminate viral charges present in the pre-contaminated water, both at medium and high concentrations, proving the effectiveness in sterilizing against bacterial and viral colonies, including *Legionella*. It shows how the use of an ultrafiltration system guarantees a safe work environment, reducing the possibility of cross-contamination both for patients and for healthcare professionals.

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| Virus strain | Viral titer (TCID₅₀⁄25 µl) | Initial titer (TCID₅₀/25 µl) | White (TCID₅₀/25 µl) | Filtered (TCID ₅₀ /25 µl) | P-value |
|--------------|-------------------------------|---------------------------------|-------------------------|---|---------------|
| Cox B5 | 10 ⁶ | 3.16 x 10 ¹ ± 1.8 | 0 | 0 | P < 0.01 [**] |
| Cox B5 | 10 ⁵ | 1.58 x 10 ¹ ± 1.1 | 0 | 0 | P < 0.01 [**] |

*: p < 0.05; **: p < 0.01.

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Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

Conceptualization: AS; methodology: AS, GM, SO, LF, DCT; supervision: AS, GM; draft writing: AS; draft review: AS, SO, LF; validation: AS; resource: AS; software: AS, LE.

References

- [1] Ma'ayeh SY, Al-Hiyasat AS, Hindiyeh MY, Khader YS. Legionella pneumophila contamination of a dental unit water line system in a dental teaching centre. Int J Dent Hyg 2008;6:48-55. https://doi.org/10.1111/j.1601-5037.2007.00280.x
- Uzel A, Cogulu D, Oncag O. Microbiological evaluation and [2] antibiotic susceptibility of dental unit water systems in general dental practice. Int J Dent Hyg 2008;6:43-7.
- Szymańska J, Sitkowska J. Bacterial contamination of dental [3] unit waterlines. Environ Monit Assess 2013;185:3603-11. https://doi.org/10.1111/j.1601-5037.2007.00269.x

- [4] Liberatore L, Murmura F, Scarano A. Bathing water profile in the coastal belt of the province of Pescara (Italy, Central Adriatic Sea). Mar Pollut Bull 2015;95:1006. https://doi.org/10.1016/j. marpolbul.2015.04.035
- Ronnie H. Etymologia: Coxsackievirus. Emerg Infect Dis [5] 2012;18:1871.
- Declerck P, Behets J, van Hoef V, Ollevier F. Detection of Le-[6] gionella spp. and some of their amoeba hosts in floating biofilms from anthropogenic and natural aquatic environments. Water Res 2007;41:3159-67. https://doi.org/10.1016/j.watres.2007.04.011
- Pankhurst CL, Coulter WA. Do contaminated dental unit water-[7] lines pose a risk of infection? J Dent 2007;35:712-20. https:// doi.org/10.1016/j.jdent.2007.06.002
- Autio KL, Rosen S, Reynolds NJ, Bright JS. Studies on [8] cross-contamination in the dental clinic. J Am Dent Assoc 1980;100:358-61. https://doi.org/10.14219/jada.archive.1980.0092
- [9] Zhang Y, Ping Y, Zhou R, Wang J, Zhang G. High throughput sequencing-based analysis of microbial diversity in dental unit waterlines supports the importance of providing safe water for clinical use. J Infect Public Health 2018;11:357-63. https://doi. org/10.1016/j.jiph.2017.09.017
- [10] Monarca S, Garusi G, Gigola P, Spampinato L, Zani C, Sapelli PL. Decontamination of dental unit waterlines using disinfectants and filters. Minerva Stomatol 2002;51:451-9.
- [11] Scarano A, Lucchina Greco C, Darcangelo C, Stilla P, Di Carlo T. Bacteriological safety of water filters for dental units: evalutation of the filtration action against S. Aureus and E. Coli. J Dent Oral Care 2018;4:13-6. https://doi.org/10.15436/2379-1705.18.1864
- [12] Glass RT, Bullard JW, Conrad RS, Blewett EL. Evaluation of the sanitization effectiveness of a denture-cleaning product on dentures contaminated with known microbial flora. An in vitro study. Quintessence Int 2004;35:194-9.

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