ORIGINAL ARTICLES AAEM

Ann Agric Environ Med 2006, 13, 313–317

ANTIFUNGAL EFFICACY OF HYDROGEN PEROXIDE IN DENTAL UNIT WATERLINE DISINFECTION*

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Szymańska J: Antifungal efficacy of hydrogen peroxide in dental unit waterline disinfection. *Ann Agric Environ Med* 2006, **13**, 313–317.

Abstract: The concentration and composition of fungal flora in dental unit waterlines (DUWL) were evaluated. For this purpose, water samples from unit reservoirs and high-speed handpieces, and biofilm from the waterline walls from units were collected. Subsequently, analogous samples from DUWL were taken before and after disinfection using agent containing hydrogen peroxide. In the examined samples, the yeast-like fungi *Candida albicans* and *Candida curvata*, *Geotrichum candidum* were found. The following species of mould were also identified: *Aspergillus amstelodami*, *Aspergillus fumigatus*, *Aspergillus glaucus* group, *Aspergillus* (=*Eurotium herbariorum*) *repens*, *Citromyces* spp., *Penicillium* (*glabrum*) *frequentans*, *Penicillium pusillum*, *Penicillium turolense* and *Sclerotium sclerotiorum* (*Sclerotinia sclerotiorum*). Before disinfection, *Candida curvata* and *Candida albicans* constituted the greatest proportion of the total fungi in the reservoirs water; in the water of handpieces - *Candida albicans* and *Aspergillus glaucus* group; and in the biofilm samples - *Aspergillus glaucus* group and *Candida albicans*. After disinfection, in all 3 kinds of samples, *Candida albicans* prevailed, constituting from 31.2-85.7% of the total fungi. The application of agent containing hydrogen peroxide caused a significant decrease both in the number of total fungi and individual fungal species, which confirms the product effectiveness in fungal decontamination of DUWL.

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Key words: dentistry, DUWL, fungi, decontamination, hydrogen peroxide.

INTRODUCTION

The quality of dental unit water is extremely important both for patients and for a dental team who are regularly exposed to microorganisms in water flowing from handpieces. The aim of the study was to assess mycological contamination of dental unit waterlines (DUWL) by determining the concentration and qualitative composition of fungal microflora in DUWL, and evaluation of the influence of a disinfecting product, agent containing hydrogen peroxide, on the mycological quality of DUWL.

MATERIAL AND METHODS

The study included 25 dental units located in public dental clinics. Water samples from unit reservoirs, water samples flowing from high-speed handpieces and samples of the biofilm formed on the inside walls of DUWL were collected from each unit. Water samples were examined by the plate dilution method. 1 ml aliquots of the aseptically collected and diluted samples were spread on a malt agar medium. For examining of the DUWL biofilm, a 15 mm-long fragment of the tubing was aseptically taken from each unit. The tube fragments were immersed

Received: 6 September 2006
Accepted: 8 October 2006
* A part of the study was presented as a poster at the 16th Congress of the International Society for Human and Animal Mycology, Paris, France, 25-29 June 2006

in 2 ml of the sterile buffered solution of physiological salt with calcium chloride and magnesium chloride, and shaken in order to obtain fungal suspension. The solution was inoculated in the same way as water samples. The malt agar plates were subsequently incubated for 4 days at 30ºC, and 4 days at 22ºC. The prolonged incubation at lower temperatures was aimed at isolating as wide a spectrum of fungi as possible. After incubation, the colonies were counted and identified to species level on the basis of colonies morphology and microscopic preparations according to generally accepted standards [3, 7, 8, 15]. The concentration of fungi was expressed as colony forming units (cfu) per 1 ml. The analogous samples from DUWL were taken twice - before disinfection, and on the 15th day after a disinfecting procedure.

Application of a disinfecting procedure. The disinfectant used was agent containing 6% hydrogen peroxide whose action is enhanced by silver ions. The product is designed for the use in dental surgeries and admitted to trading in Poland. The disinfectant was used according to the manufacturer's instructions to obtain a desired hydrogen peroxide concentration. DUWL disinfection procedure comprised 2 stages. First, DUWL underwent intensive disinfection with 0.25% hydrogen peroxide which was kept present in all the waterlines elements for 30 min due to a continual flow of water from the reservoir to the handpieces - water was flushed through all unit handpieces. The second stage consisted in the constant presence of 0.02% hydrogen peroxide in DUWL.

Statistical analysis. The obtained results were analysed with Wilcoxon test using Microsoft Excel 2000 and Statistica 5.1. The assumed error risk was 5%.

RESULTS AND DISSCUSION

Fungi in DUWL before disinfection. Fungi were found in 12 water samples from reservoirs (48%), in 16 water samples from the handpieces (64%), and in 11 biofilm samples (44%). The concentration of total fungi at the individual operative sites ranged from 0-645 × 10^1 cfu/ml in the reservoirs water, from 0-375 × 10^1 cfu/ml in the water flowing from high-speed handpieces, and from 0-311.39 cfu/mm^2 in the biofilm samples (Tab. 1). In the examined DUWL samples, the yeast-like fungi *Candida albicans*, *Candida curvata* and *Geotrichum candidum* were found. The following species of moulds were also identified: *Aspergillus amstelodami*, *Aspergillus fumigatus*, *Aspergillus glaucus* group, *Aspergillus repens*, *Citromyces* spp., *Penicillium* (*glabrum*) *frequentans*, *Penicillium pusillum*, *Penicillium turolense* and *Sclerotium sclerotiorum* (*Sclerotinia sclerotiorum*) (Tab. 2, 3). *Candida curvata* and *Candida albicans* constituted the greatest proportion of the total fungi in the reservoirs water; in the water of handpieces - *Candida albicans* and

Table 1. Concentration of total fungi in DUWL at individual operative sites before and after DUWL disinfection.

Site	Before disinfection			After disinfection		
	Reservoir	Handpiece	Biofilm	Reservoir	Handpiece	Biofilm
	cfu/ml	cfu/ml	cfu/mm^2	cfu/ml	cfu/ml	cfu/mm^2
1	700	1,130	0	40	40	0
2	30	700	0	0	0	0
3	30	1,000	0	0	20	0
4	80	110	5.66	10	30	0.85
5	540	860	166.74	20	10	0.85
6	780	940	49.26	30	10	1.13
7	220	960	237.23	10	10	1.42
8	0	820	0	0	0	0
9	1,290	0	245.44	30	0	1.13
10	0	0	0	0	0	0
11	0	0	0	0	0	0
12	0	0	0	0	0	0
13	0	710	5.10	0	0	0
14	0	200	0	0	0	0
15	0	0	6.79	0	0	0
16	0	80	0	0	0	0
17	0	1,280	0	0	0	0
18	10	0	28.31	0	0	0.28
19	110	240	28.31	0	0	0
20	0	160	0	0	0	0
21	0	0	0	0	0	0
22	6,450	0	0	0	0	0
23	0	1,520	0	0	40	0
24	10	0	1.70	0	0	0
25	0	3,750	311.39	0	0	0
Mean	410	578.4	43.44	5.6	6.4	0.23

Table 2. Fungi isolated from DUWL at individual operative sites before DUWL disinfection.

RESERVOIR
Yeast-like fungi:
Candida albicans (1, 2, 3, 4, 5, 6, 9, 19), *Candida curvata* (9, 22, 24)
Mould fungi:
Aspergillus glaucus group (5, 6, 7, 9), *Sclerotium sclerotiorum* (18, 19)

HANDPIECE
Yeast-like fungi:
Candida albicans (1, 13, 14, 16, 17, 19, 20, 23), *Candida curvata* (25), *Geotrichum candidum* (2)
Mould fungi:
Aspergillus fumigatus (5), *Aspergillus glaucus* group (2, 5, 6, 7, 8), *Aspergillus repens* (1), *Citromyces* spp. (3), *Penicillium* (*glabrum*) *frequentans* (1, 4), *Penicillium pusillum* (4), *Sclerotium sclerotiorum* (25)

BIOFILM
Yeast-like fungi:
Candida albicans (5, 6, 7, 9, 13, 15), *Candida curvata* (9, 24, 25), *Geotrichum candidum* (19)
Mould fungi:
Aspergillus amstelodami (7), *Aspergillus fumigatus* (6), *Aspergillus glaucus* group (5, 6, 7, 9), *Penicillium pusillum* (4), *Penicillium turolense* (18), *Sclerotium sclerotiorum* (25)

Aspergillus glaucus group, and in the biofilm samples - *Aspergillus glaucus* group and *Candida albicans* (Tab. 3).

Fungi in DUWL after disinfection. Fungi were found in 6 water samples from the reservoirs (24%), in 7 water samples from handpieces (28%), and in 6 biofilm samples (24%). The concentration of total fungi in the studied water samples, both from the reservoirs and from handpieces ranged from 0-4 × 10^1 cfu/ml, and in the biofilm samples - from 0-5 × 10^1 cfu/ml (Tab. 1). The same species of fungi were present in DUWL as before disinfection protocol, except for yeast-like fungi *Candida curvata*, *Geotrichum candidum*, and the following mould fungi: *Aspergillus amstelodami*, and *Sclerotium sclerotiorum*. In all 3 kinds of DUWL samples, *Candida albicans* prevailed, constituting from 31.2-85.7% of the total fungi (Tab. 3).

The obtained results were analysed statistically. The descriptive statistics are presented in Table 4. The Wilcoxon test showed that the disinfecting method used caused a significant decrease in concentration of the total fungi in the reservoir water, in the water flowing from a high-speed handpiece, and in the biofilm. Considering the effect of the DUWL disinfecting method used on yeast-like and mould fungi, a significant decrease in concentration of both kinds of fungi was found (Tab. 5). This confirms antimycotic effectiveness of a disinfecting procedure with the use of hydrogen peroxide.

It is believed that there are 2 sources of microbiological contamination of dental unit waterlines. The first may be municipal water piped into the dental unit (directly - in the case of an open system water supply, or after distillation - in the case of a closed system water supply). The second source are patients, i.e. a suck-back of their saliva into the line due to the lack of antireaction valves. Pederson *et al.* [13] believed that *Candida* species found in DUWL, which belong to the commonly reported oral flora, were most likely derived from suck-back events. Reaspiration of fluid from the oral cavity occurs when a

Table 4. Mean concentration of fungi before and after DUWL disinfection (cfu/ml).

Fungi	Before disinfection			After disinfection		
	Mean	Min	Max	Mean	Min	Max
Yeast-like fungi						
reservoir (cfu/ml)	374,4	0	6450	4,8	0	40
handpiece (cfu/ml)	197,2	0	1520	2	0	40
biofilm (cfu/mm^2)	18,07	0	203,82	0,11	0	0,57
Mould fungi						
reservoir (cfu/ml)	35,6	0	220	0,8	0	10
handpiece (cfu/ml)	381,2	0	1100	4,4	0	30
biofilm (cfu/mm^2)	25,36	0	195,33	0,11	0	0,85
Total fungi identified						
reservoir (cfu/ml)	410,0	0	6450	5,6	0	40
handpiece (cfu/ml)	578,4	0	3750	6,4	0	40
biofilm (cfu/mm^2)	43,44	0	311,39	0,23	0	1,42

mean - mean concentration for all sites, min - minimum concentration at a site, max – maximum concentration at a site

Table 5. Statistical analysis of disinfection effect on fungi concentration in DUWL (Wilcoxon test).

Fungi	Significance	p
Yeast-like fungi – reservoir	**	0,005065
Yeast-like fungi – handpiece	**	0,005065
Yeast-like fungi – biofilm	**	0,007690
Mould fungi – reservoir	*	0,027715
Mould fungi – handpiece	**	0,007690
Mould fungi – biofilm	*	0,017966
Total identified fungi – reservoir	**	0,002220
Total identified fungi – handpiece	***	0,000438
Total identified fungi – biofilm	**	0,003348

p - significance level for Wilcoxon test; * - $p < 0.05$, ** - $p < 0.01$, *** - $p < 0.001$.

Table 3. Mean concentration of fungal species in DUWL before and after DUWL disinfection.

Fungal species	Before disinfection			After disinfection		
	Reservoir	Handpiece	Biofilm	Reservoir	Handpiece	Biofilm
	cfu/ml	cfu/ml	cfu/mm^2	cfu/ml	cfu/ml	cfu/mm^2
Candida albicans	90.0	168.8	11.19	4.8	2.0	0.11
Candida curvata	284.4	20.4	5.75	0.0	0.0	0
Geotrichum candidum	0.0	8.0	1.13	0.0	0.0	0
Aspergillus amstelodami	0.0	0.0	1.13	0.0	0.0	0
Aspergillus fumigatus	0.0	4.0	1.13	0.0	0.4	0.02
Aspergillus glaucus group	31.2	159.2	14.49	0.8	0.8	0.05
Aspergillus repens	0.0	4.0	0	0.0	0.4	0
Citromyces spp.	0.0	40.0	0	0.0	0.8	0
Penicillium (*glabrum*) *frequentans*	0.0	40.4	0	0.0	0.8	0
Penicillium pusillum	0.0	4.0	0.23	0.0	1.2	0.03
Penicillium turolense	0.0	0.0	1.13	0.0	0.0	0.01
Sclerotium sclerotiorum	4.4	129.6	7.25	0.0	0.0	0
Total	410.0	578.4	43.43	5.6	6.4	0.22

negative pressure is generated on stopping equipment. It should be stressed that the examined units were manufactured over 10 years ago, and are earlier-generation devices without antireaction values. Moreover, the safety principles for the work in the patient's oral cavity with the use of dental unit handpieces require the handpiece to remain in the oral cavity until the device has stopped working (whirling). The handpiece is withdrawn from the patient's oral cavity at a standstill, i.e. when a negative pressure is generated; thus, without antireaction valves, a suck-back of fluid from the patient's oral cavity was possible.

In present study, *Candida albicans* fungi were present in all types of samples taken from DUWL both before and after disinfection. The latter significantly decreased their concentration, however, this fungal species prevailed in the identified DUWL fungal microflora. It should be underlined that *Candida albicans* is the species of the greatest clinical importance. *Candida albicans* is a dimorphic, opportunistic pathogenic fungus that grows either in the yeast form or as hyphae. It is a commensal resident on the mucosal surfaces and can cause host damage (candidiasis) by mechanisms mediated both by host (predisposing factors) and by fungus (virulence factors). *Candida albicans* can cause different types of infections ranging from superficial to systemic candidiasis. The latter remains a leading infectious cause of morbidity and mortality in critically ill and/or severely immunocompromised patients [5].

The enhanced pathogenicity of *Candida albicans* strains is related to their ability to occur in 2 forms: yeast-like – blastospora, and filamentous - mycelium. *Candida albicans* strains are extremely adhesive to host epithelial cells and are characterised by high pathogenicity. Biofilm forming by *Candida* fungi plays an essential role in pathogenesis of infections caused by these microorganisms. *Candida* biofilm may form on the surface of plastics, such as methyl polymethacrylate, elastomeric silicone, or vinyl polychloride. Metabolic activity of *Candida albicans* biofilm is much higher than that of the biofilm formed by other species of *Candida* genus. *Candida albicans* colonises the surfaces, developing biofilms that are extremely resistant to most antifungal agents, and synthesis of glycosylated mannoproteins is an important factor in the development and integrity of *Candida albicans* biofilms [14].

A previous paper presented the results of a mycological analysis of the bioaerosol forming during conservative treatment with a dental unit high-speed handpiece. Air samples were taken simultaneously from units analogous to those for which mycological assessment of water and biofilm, described in this article, was performed. The bioaerosol had a different mycological composition than the one found in DUWL water and biofilm samples [18]. It is worth noting that fungi of *Candida* genus were identified only in the DUWL samples.

Fungi of *Aspergillus* genus may cause opportunistic mycoses - aspergillosis. Infections with *Aspergillus* fungi concern mainly lungs. The infection resulting from inhaling *Aspergillus* spores may cause bronchial asthma in a patient allergic to the antigen of these fungi. In immunocompromised patients, the infection may take an invasive form and may lead, among others, to septicemia, aspergillosis of endocardium, central nervous system, skin and subcutaneous tissue. *Aspergillus fumigatus* is known as the primary cause of invasive aspergillosis [10] and a fungal pathogen causing allergic alveolitis, asthma, pulmonary aspergillosis and possibly mycotoxicoses. According to investigations by Seidler *et al.* [16], *Aspergillus fumigatus* is able to form a biofilm-like structure in vitro that is similar to biofilms of *Candida* spp.

The fungi of *Penicillium* genus are characterised with strong allergenic properties and may cause bronchial asthma, asthma with lung eosinophilia (AEP), and allergic rhinitis [4, 6]. The latest communications indicate that in dental unit waterlines fungi are found both in water and in the biofilm. Fungal elements were found in 28% of the water samples from air-water syringes and in 36% of the water samples from high-speed handpieces; filamentous fungi were the most frequently isolated fungi [11]. Earlier studies identified the presence of the following fungi in DUWL: *Phoma* spp., *Penicillium* spp., *Cladosporium* spp., *Alternaria* spp., *Scopulariopsis* spp. [12]. In DUWL water, were noted also *Fusarium* spp. [20] and some yeasts [2]. A study by Barbeau *et al.* [1] showed that in the biofilm suspension, yeast-like microorganisms were present, but constituted less than 0.1% of the total microflora colonising DUWL polyurethane tubes. The presence of fungal microflora in the biofilm was found also by other researchers [9, 19].

CONCLUSION, SIGNIFICANCE AND IMPACT OF THE STUDY

Among the fungal flora contaminating DUWL, fungi of the *Candida* genus prevailed: *Candida albicans* and *Candida curvata*. The general concentration of fungi was lower in the water from a reservoir which is the water source in a unit, in comparison with the water flowing from a highspeed handpiece. At the same time, fungi were not found in the reservoir water from ca. 50% of the operative sites. It should be noted that in the reservoirs water, the diversity of identified fungal species is two times lower than in the water flowing from high-speed handpieces. The latter suggests that the biofilm present on the walls of the waterlines may be a source of mycological contamination of DUWL, and confirms the fact that in the biofilm there are good conditions for fungal multiplication.

Application of hydrogen peroxide caused: 1. decrease in the number of operative sites where fungi were found; 2. significant decrease both in the number of the total fungi and fungi at individual operative sites in all types of DUWL samples (reservoir water, water flowing from a high-speed handpiece, biofilm); 3. reduction in the concentration of *Candida albicans*, which prevailed

before and after application of a disinfecting procedure, and complete elimination of *Candida curvata*.

The use of dental unit waterlines decontamination protocol is an important way to control the appropriate quality of water used in dental treatment, and thus to minimize the risk resulting from exposure to waterborne pathogens. Decontaminating effects of hydrogen peroxide on the bacterial component of the biofilm present on DUWL walls were demonstrated in an earlier study [17]. Thus, the use of the analysed agent, which shows decontamination effectiveness both for bacteria and fungi, fulfills its task as a method of microbiological quality control of both DUWL water and DUWL biofilm.

A long-term assessment of the use of hydrogen peroxide in the conditions of everyday dental clinical practice would be interesting. It should be stressed that DUWL biofilm samples, both for bacteriological and mycological examination, were taken in clinical settings, from units actually used to treat patients. The studied DUWL biofilm was exposed both to a disinfection process and the influence of various factors present in a dental surgery.

Acknowledgements

I am grateful to Dr. Ewa Krysińska-Traczyk for performing the mycological investigation.

REFERENCES

1. Barbeau J, Avezard C, Faucher E, Zalzal S, Prévost AP: Biofilms in dental unit waterlines. Ultrastructural and cytochemical analysis. *Cells and Materials* 1997, **7**, 135-146.
2. Barbeau J, Tanguay R, Faucher E, Avezard C, Trudel L, Cote L, Prevost AP: Multiparametric analysis of waterline contamination in dental units. *Appl Environ Microbiol* 1996, **62**, 3954-3959.
3. Barron GL: *The Genera of Hyphomycetes from Soil*. Williams & Wilkins, Baltimore 1968.
4. Dutkiewicz J, Jabłoński L: *Occupational Biohazards*, 1st ed. PZWL, Warsaw 1989 (in Polish).
5. Gil C: *Candida albicans* - host interaction: Insights from proteomics. **In:** *Final Program and Presentation Summaries. The 16th Congress of the International Society for Human and Animal Mycology, Paris, France, 25-29 June 2006*, 175-176. ISHAM, Paris 2006.
6. Lacey J, Dutkiewicz J: Bioaerosols and occupational lung disease. *J Aerosol Sci* 1994, **25**, 1371-1404.
7. Larone DH: *Medically Important Fungi: A Guide to Identification*. American Society for Microbiology, Washington, D.C. 1993.
8. Litvinov MA: *Opredelitel' Mikroskopicheskikh Pochvennykh Gribov*. Izd. Nauka, Leningrad 1967.
9. Murdoch-Kinch CA, Andrews NL, Atwan S, Jude R, Gleason MJ, Molinari JA: Comparison of dental water quality management procedures. *J Am Dent Assoc* 1997, **128**, 1235-1243.
10. Nierman WC, Pain A, Anderson MJ, *et al.* Genomic sequence of the pathogenic and allergenic filamentous fungus *Aspergillus fumigatus*. *Nature* 2005, **438**, 1151-1156.
11. Nikaeen M, Hatamzadeh M, Mirhendi S, Sabzevari Z, Zareh O: Microbial quality of water in dental unit waterlines. **In:** *Abstract Book. The 16th Congress of the International Society for Human and Animal Mycology. Paris, France, 25-29 June 2006*, P-0525. ISHAM, Paris 2006.
12. Pankhurst CL, Johnson NW: Microbial contamination of dental unit waterlines: scientific argument. *Inter Dent J* 1998, **48**, 359-368.
13. Pederson ED, Stone ME, Ragain JC, Simecek JW: Waterline biofilm and the dental treatment facility: a review. *Gen Dent* 2002, **50**, 190-195.
14. Ramage G, Coco B, Gow N: Glycosylated mannoproteins are essential to *Candida albicans* biofilm integrity and development. **In:** *Abstract Book. The 16th Congress of the International Society for Human and Animal Mycology. Paris, France, 25-29 June 2006*, P-0732. ISHAM, Paris 2006.
15. Ramirez C: *Manual and Atlas of the Penicillia*. Elsevier, Amsterdam 1982.
16. Seidler M, Salvenmoser S, Müller F: Production of biofilm-like matrix by *Aspergillus fumigatus* on polystyrene and bronchial epithelial cells. In: *Abstract Book. The 16th Congress of the International Society for Human and Animal Mycology. Paris, France, 25-29 June 2006*, P-0323. ISHAM, Paris 2006.
17. Szymańska J: Bacterial decontamination of DUWL biofilm using Oxygenal 6. *Ann Agric Environ Med* 2006, **13**, 163-167.
18. Szymańska J: Exposure to airborne fungi during conservative dental treatment. *Ann Agric Environ Med* 2006, **13**, 177-179.
19. Williams JF, Johnston AM, Johnson B, Huntington MK, Mackenzie CD: Microbial contamination of dental unit waterlines: prevalence, intensity and microbiological characteristics. *J Am Dent Assoc* 1993, **124**, 59-65.
20. Wróblewska M, Piotrowska E: Etiologia i epidemiologia zakażeń w praktyce stomatologicznej. **In:** Łuczak M, Swoboda-Kopeć E (Eds): *Wybrane Zagadnienia z Mikrobiologii Jamy Ustnej*, 355-366. 1st ed. Wydawnictwo Czelej, Lublin 2004.