Coll. Antropol. **37** (2013) 1: 101–107
Original scientific paper

Efficacy of Ozone on Microorganisms in the Tooth Root Canal

Kristina Halbauer[1], Katica Prskalo[2], Bernard Janković[2], Zrinka Tarle[2], Vlatko Pandurić[2] and Smilja Kalenić[3]

[1] Private Dental Practice, Osijek, Croatia
[2] University of Zagreb, School of Dental Medicine, Department of Endodontics and Restorative Dentistry, Zagreb, Croatia
[3] University of Zagreb, Zagreb University Hospital Center, Department of Clinical and Molecular Microbiology, Zagreb, Croatia

A B S T R A C T

The aim of this study was to examine the effect of ozone gas on the remaining bacteria after chemomechanical instrumentation of tooth root canal. The study was carried out at the Department of Endodontics and Restorative dentistry, School of Dental Medicine, University of Zagreb. A total of 37 tooth root canals from 23 teeth (10 incisors, 2 canines, 8 premolars and 3 molars) with a diagnosis of chronic apical periodontitis (17 untreated teeth and 6 retreatments) from 20 adult patients (11 females and 9 male) were selected. Endodontic samples consisted of 74 swabs from 37 canals. The first root canal swab was taken following a completed chemomechanical instrumentation by a sterile paper point after rinsing the root canal with a sterile saline solution. The canal was dried and treated with ozone gas for 40 seconds (HealOzone, Kavo, Germany). After the ozone treatment the canal was rinsed with a sterile saline solution a second swab was taken. The swabs were stored in transport media until cultivation. Microbiological identification was performed by macromorphological, micromorphological, commercial biochemical test microbiological analysis and bacteria count. A significant decrease in the number of bacteria ($p<0.001$) was found after the ozone treatment: the total number of bacteria was 82%, 67% of aerobic and 93% of anaerobic bacteria. When analysing individually, a significant decrease was found for Streptococcus mitis and Propionibacterium acnes ($p<0.05$). The results of this study shows the efficacy of ozone on the bacterial count reduction in the root canal treatment.

***Key words**: ozone, root canal microorganisms*

Introduction

The basis of endodontic therapy lies in knowing and eliminating cause factors of the endodontic infection. Over 300 bacterial species have already been isolated from or detected in infected root canals, but no single species has been consistently found to be the major endodontic pathogen[1]. Culture and molecular studies have showed that the microbiota associated with primary endodontic infections is dominated by anaerobic bacteria and that an infected root canal can harbor from 10 to 30 bacterial species[2].

The aim of the biomechanical instrumentation is to eliminate the microorganisms from the endodontic space, to prevent the spread of infection and postoperative periapical reinfecton. Chemomechanical instrumentation of root canal include intracanal cleaning, enlarging and shaping root canal walls, chemical irrigation and the use of root canal filling materials. Modern methods of microbial control are directed towards the use of intracanal medicaments that act against different types of bacteria (aerobic, anaerobic and microaerophiles) and also affect cell wall synthesis or alter the cytoplasmic membrane's permeability and interfere with protein synthesis or chromosomal replication[3]. Microorganisms can survive the effects of chemomechanical instrumentation in approximately 40–70% of the cases[4,5]. There are a few circumstances that may be responible for this: inappropriate mechanical root canal dentin debridement, invasion of dentinal tubules by root canal bacteria, low quality of apical shaping, possible biofilm formation[6], bacterial resistance to endodontic procedures, type and concentration of irrigants and intracanal filling as well as frequency of irrigation. In this context, the capability of

Received for publication September 9, 2012

microorganisms surviving in a limited nutrient root canal environment made by anti-microbial therapy is of special interest. The data from different screening studies after instrumentation and intracanal filling show reduction in the number of bacteria that were still present at the time of the root filling[7]. The most common are Gram negative anaerobic rods (*Fusobacterium nucleatum*, *Prevotella spp*., *Campylobacter rectus*) and Gram-positive bacteria (Streptococci, Lactobacilli, Staphylococci, *Enterococcus faecalis*, *Propionibacterium spp*., *Actinomyces spp*., *Bifidobacterium spp*., *Eubacterium spp*.)

Apart from the well known NaOCl solutions, EDTA, calcium-hydroxide and chlorhexidine, ozone has recently been used in root canal disinfection. Ozone is a very reactive, unstable, toxic and irritating blue gas with three oxygen atoms and strong antimicrobial activity. Artificially it is produced by ozone generator with UV, cold plasma or corona discharge system. Its strong oxidant activity destroys bacteria, fungi and viruses by attacking cells and the cytoplasmatic membrane[8]. Ozone rapidly reacts with the biological tissue and initiates the production of reactive oxygen species (ROS) and products of lipid peroxidation (LOP)[9].

The use of ozonated water in the treatment of endodontic infections has been suggested[10,11]. A recent study on the use of ozonized oil and calcium hydroxide paste on common bacterial species in periradicular diseases, shows that ozonized oil has been the most effective treatment[12]. Cardoso et al. found a decrease in the number of *Candida albicans* and *Enterococcus faecalis* in root canal irrigated with ozonated water[13]. Silveira et al. proved a root canal treatment using ozonizated oil was successful in 77% of the cases[14].

The purpose of this study was to evaluate the antimicrobial efficiency of ozone gas on bacteria in human tooth root canals *in vivo*.

Material and Methods

Study population

Ethical approval was obtained from the Ethics Committe of the School of Dental Medicine, University of Zagreb, Croatia. Each patient signed a consent form for dental examination, root canal treatment, ozone treatment and microbiological sampling that were done at the Department of Endodontics and Restorative Dentistry, School of Dental Medicine, Zagreb and the Department of Microbiology, Zagreb University Hospital Center.

All 20 patients were referred to the Department of Endodontics and Restorative Dentistry, School of Dental Medicine, Zagreb for endodontic therapy. After the examination and diagnostic procedures of a total 37 root canals from 23 teeth (10 incisors, 2 canines, 8 premolars and 3 molars) with a diagnosis of chronical apical periodontitis (17 untreated teeth and 6 retreatments) from 20 adult patients (11 females and 9 males) were selected for the study. Diagnosis for each patient was recorded.

Tooth preparation

The access cavity was prepared with sterile high-speed diamond burs (Komet Dental, Gebr. Brasseler GmbH & Co. KG, Lemgo, Germany) under irrigation with distilled water spray. In case of previous endodontic treatment, the old restoration and the root canal filling were removed before instrumentation. After isolation with a rubber dam and before entering the pulp chamber, the crown and surrounding working area were disinfected with a 2.5% sodium hypochlorite solution (NaOCl) to ensure an aseptic operative condition. The pulp chamber was accessed by sterile round burs and the content was removed with an excavator. The root canal working length was determined by an electronic apex locator ES-02 (Artronic, Zagreb, Croatia). The canals were instrumented with a standard hand step-back technique up to a master apical file (K file, Dentsplay Maillefer, Ballaigues, Switzerland) of at least three sizes larger than the initial apical file. Mechanical instrumentation was simultaneously followed by copious 2.5% NaOCl irrigation. After cleaning and shaping, the root canal was finally rinsed with a sterile saline solution (Pliva, Zagreb, Croatia). Microbiological samples were taken after the chemomechanical instrumentation and after the additional ozone gas root canal disinfection.

Sample collection

Each sample had two swabs taken for microbiological analysis. The first swab was taken after completing chemomechanical instrumentation and rinsing the root canal with a sterile saline solution. The first swabs represented the control group of the study.

A sterile absorbent paper point (DiaDent, Burnaby, Canada) sized as master apical file was introduced into the root canal in full working length and left to soak up the liquid in the canal. After 20 seconds the paper point was immediately transferred into the transport media for anaerobes WMGA.

The canal was dried with sterile paper points and the ozone was applied for 40 seconds by a HealOzone delivery system (KaVo, Germany). HealOzone is a medical electronic device that generates ozone gas from the air. The HealOzone device is a closed system of ozone delivery which does not allow dispersal of the ozone in the environment. The disposable sterile silicone cup forms a seal around the tooth and a vacuum conducts the ozone through the handpiece into the cap at a concentration of 2,100 ppm ± 10%. The ozone gas is refreshed in the cup at a flow rate of 615 cm^3/minute by changing the volume of gas inside the cup over 300 times every second.

After the ozone treatment the root canal was rinsed with a sterile saline solution and a second swab was taken in the same manner as previously described. The second swabs represented the tested group in the study.

Finally the root canals were obturated by the lateral condensation technique. The pulp chamber was sealed with a temporary cement until final restoration.

TABLE 1
DESCRIPTIVE STATISTICS: THE NUMBER OF BACTERIA AND STATISTICAL TEST OF DISTRIBUTION NORMALITIES

	$\overline{X}$ number of bacteria	SD*	95% CI†	Median	IQR‡	Min.	Max.	N	K-S, p
Aerobic									
Before ozone	12.3	±27.65	2.9–21.6	5.5	2–9.7	0	159	37	<0.001
After ozone	2.2	±3.18	1.1–3.3	1.0	0–3	0	12	37	0.022
Anaerobic									
Before ozone	2.4	3.61	1.3–3.6	0.0	0–5	0	12	37	0.006
After ozone	0.2	0.55	0.0–0.3	0.0	0	0	3	37	<0.001
Total									
Before ozone	14.4	29.43	4.6–24.3	7.0	2.5–14	0	172	37	<0.001
After ozone	2.3	3.39	1.2–3.4	1.0	0–3	0	12	37	0.010

* Standard Deviation; † 95% Confidence Interval for Mean; ‡ Interquartile Range; K-S: Kolmogorov Smirnov test of normality of the distribution; p is Monte Carlo Exact

Isolation and identification of microorganisms

Transport media were delivered at room temperature to the microbiological laboratory within two hours. The samples were planted onto nonselective and selective enriched media. The nonselective media were used for isolation of facultative anaerobic bacteria and strictly anaerobic bacteria (BBLTM Blood Agar Base Infusion Agar, Beckton Dickinson and Co., Sparks, Maryland, USA). Strictly anaerobic pathogenic bacteria were isolated on BBLTM Columbia Agar Base (Beckton Dickinson and Co., Sparks, Maryland, USA). In order to acquire anaerobic conditions during cultivation of anaerobic bacteria the Das Pak TMEZ Anaerobe Container System (Beckton Dickinson and Co., Sparks, Maryland, USA) was used.

The media were incubated at 35 °C for 24–72 hours, or for seven days, depending on the types of media.

Bacteria were counted and identified by macromorphological and micromorphological methods and biochemical identification with commercial biochemical tests, API® 20 A (BioMerieux, Marcy l'Etoile, France) for strict anaerobes and API® 20 Strep for *Streptococcus spp.* (BioMerieux, Marcy l'Etoile, France).

Results

The mean number of aerobic, anaerobic and total bacteria (aerobic and anaerobic) before and after the ozone treatment is presented in Table 1. Distributions of all original variables were statistically significantly different from normal distributions. Kolmogorov-Smirnov one sample test varied from <0.001 up to 0.022. Therefore the nonparametric Wilcoxon test was used for the assessment of differences in the median number of bacteria before and after the additional treatment with ozone.

As presented in Table 2, the decrease in number of bacteria after the treatment with ozone was highly statistically significant: Z=–4.609, p<0.001 for aerobic, and Z=–3.731, p<0.001 for anaerobic bacteria. A decrease in the total number of bacteria of 82% (Z=–4.826, p<0.001) was found: 67% of aerobic, or 93% of anaerobic bacteria respectively.

Tables 3 and 4 present the number and percentage of samples with aerobic or anaerobic bacteria before and after ozone treatment. The total sum exceeds 100% due to multiple responses. Only the swabs with some kind of bacteria are shown. There were 5 swabs (14%) with no aerobic bacteria before the ozone treatment, and 15 (42%) with no aerobic bacteria after the ozone treatment. Before the ozone treatment there were 20 (53%) swabs with no anaerobic bacteria and after the ozone treatment

TABLE 2
TEST OF THE STATISTICAL SIGNIFICANCE OF DECREASE IN NUMBER OF BACTERIA AFTER TREATMENT WITH OZONE

	Median diff.	Wilcoxon Signed Ranks Test			
		N	Z	p†	Effect size‡
Aerobic					
Before ozone		37	–4.609	<0.001	–0.76
After ozone	67%	37			
Anaerobic					
Before ozone		37	–3.731	<0.001	–0.61
After ozone	93%	37			
Total					
Before ozone		37	–4.826	<0.001	–0.79
After ozone	82%	37			

Median diff – median difference in number of bacteria from the previous phase; it was calculated for each case as (initial number of bacteria-number of bacteria in the given phase)/ the initial number of bacteria. Final median for all cases was calculated on this data. † Monte Carlo test of statistical significance; ‡ Effect size was calculated as: r = Z/sqrt(n)

TABLE 3
NUMBER AND PERCENTAGE OF SAMPLES WITH EACH TYPE OF AEROBIC BACTERIA BEFORE AND AFTER THE OZONE TREATMENT

	Before ozone		After ozone		Sig. (McNemar test, exact p)
	N	%	N	%	
Streptococcus mitis	14	(45)	7	(33)	0.039
Neisseria saprophytica	10	(32)	6	(29)	>0.05
Staphylococcus epidermidis	7	(23)	6	(29)	>0.05
Corynebacterium species	6	(19)	5	(24)	>0.05
Enterococcus faecalis	4	(13)	1	(5)	>0.05
Streptococcus oralis	3	(10)	2	(10)	>0.05
Streptococcus sanguinis	2	(6)	2	(10)	>0.05
Beta hemolit streptococcus ser. gr. A	2	(6)			
Enterobacter species	2	(6)	2	(10)	>0.05
Bacillus species	2	(6)			
Klebsiella pneumoniae	2	(6)	1	(5)	>0.05
Gemella morbilliorum	2	(6)			
Streptococcus milleri II	2	(6)			
Staphylococcus aureus	1	(3)	1	(5)	>0.05
Streptococcus constellatus	1	(3)	1	(5)	>0.05
Lactobacillus species	1	(3)	1	(5)	>0.05
Pseudomonas aeruginosa	1	(3)	1	(5)	>0.05
Lactococcus lactis cremoris	1	(3)	2	(10)	>0.05
Haemophillus species	1	(3)			
Streptococcus mutans	1	(3)			
Staphylococcus saprophyticus	1	(3)			
Streptococcus acidominimus	1	(3)			

TABLE 4
NUMBER AND PERCENTAGE OF SAMPLES WITH EACH TYPE OF ANAEROBIC BACTERIA BEFORE AND AFTER THE OZONE TREATMENT

	Before ozone		After ozone		Sig.(McNemar test, exact p)
	N	%	N	%	
Propionibacterium acnes	7	(39)	2	(40)	0.031
Prevotella melaninogenica	5	(28)	2	(40)	>0.05
Veillonella parvula	4	(22)			
Bifidobacterium adolescetis	2	(11)			
Streptococcus sanguis	1	(6)			
Staphylococcus epidermidis	1	(6)	1	(20)	>0.05
Fusobacterium species	1	(6)			
Prevotella intermedia	1	(6)			
Fusobacterium nucleatum	1	(6)			
Prevotella oralis	1	(6)			
Bacteroides fragilis	1	(6)			
Actimomyces meyeri	1	(6)			
Lactobacillus fermentum	1	(6)			
Gamella morbillorum	1	(6)			

there was 34 (89%) with no anaerobic bacteria. For both types of bacteria, aerobic and anaerobic, the McNemar test shows that there is statistically significant fewer swabs with any type of bacteria after the ozone treatment compared to those before the ozone treatment ($p<0.001$).

When analysing bacteria individually, a statistically significant decrease is observed only for *Streptococcus mitis* ($p<0.05$) and *Propionibacterium acnes* ($p<0.05$).

Discussion

This study shows the advantage of the additional root canal treatment using the HealOzone device for ozone production on the bacterial count reduction in relation to the conventional chemomechanical treatment. Efficacy of the ozone treatment was confirmed by the decrease the total number and in the number of aerobic and anaerobic bacteria separately.

Several studies[15–19] and this one as well, before the ozone treatment, proved inefficiency of intracanal rinsing solution and medicaments in combination with mechanical treatment to eliminate the root canal bacteria. Microbiological findings depend on tooth diagnosis selection, oxygen presence, bacterial nutrition needs, residual effect of the medicaments, inoculation time, selective growing media, swab taking methods and bacterial detection technique. The use of more sensitive bacteria detection methods, such as the qPCR reveals a far wider range of bacterial types and gives more precise microbiological findings concerning count, type and viability of bacterial cells.

Completed chemomechanical instrumentation of the canal was a selection criterium for intracanal swab taking. Thereby, the selection procedures, methods and root canal swab taking techniques used in similar researches were applied[20–22]. Their investigations found disinfection by different concentrations of NaOCl solution, alcohol, H_2O_2 and iodine solutions and used different transport media (TRF, WMGA II). Swab taking lasted 10–60 seconds and samples were submitted for analysis within 15 minutes to 24 hours.

In our study, the principles of total asepsis and NaOCl irrigation solution during the chemomechanical instrumentation were used. Swab taking lasted for 20 seconds, and special WMGA transport medium was used to transport swabs within two hours to the microbiological laboratory. Bacteria cultivation was performed on special selective media for cultivating anaerobic bacteria.

As compared to the other studies, the results of this study showed a small number of bacteria. It may be the result of a proper chemomechanical instrumentation or the residual effect of intracanal medicament as mentioned by Lambrianidis et al.[23]. There is a possibility that paper point was not long enough in the canal so there was an insufficient amount of bacteria for cultivation. The difference in the results is possible due to the difference in sample transportation time. Anaerobic bacteria are highly sensitive to oxygen so there is a possibility of bacteria dying even before cultivation and consequently unable of detect.

Regarding the bacteria isolated in this study, the results of microbiological analysis after chemomechanical instrumentation showed similar results in the number and types of bacteria as in other similar studies[21,22]. Significant decrease in the number of bacteria was found ($p<0.001$ for aerobic and $p<0.001$ for anaerobic bacteria) after the ozone treatment (Table 1). As presented in Table 2 the average number of bacteria decreased after the ozone treatment by 67% for aerobic and 93% for anaerobic bacteria. Concerning both aerobic and anaerobic bacteria, there is a significant decrease in their number by 82% ($p<0.001$).

In this study, the increase in the number of samples without aerobic bacteria after the ozone treatment was statistically significant. Before the ozone treatment 14% of the samples were without aerobic bacteria, whereas after the ozone treatment 42% of the samples were without bacteria. The most common bacteria before the ozone treatment were *Streptococcus mitis*, *Neisseria* (saprofitic), *Staphylococcus epidermidis*, *Corynebacterium species*, *Enterococcus faecalis* and *Streptococcus oralis*. After the ozone treatment only statistically significant reduction of *Streptococcus mitis* was observed (Table 3).

Initially, 53% of the samples were without anaerobic bacteria while after the ozone treatment 89% of the samples were without anaerobic bacteria, which shows statistically significant decrease. The most common anaerobic bacteria before the ozone treatment were *Propionibacterium acnes, Prevotela melaninogenica, Veillonella parvula and Bifidobacterium adolescentis*. After the ozone treatment, statistically significant difference was found for *Propionibacterium acnes* (Table 4). *Propionibacterium acnes* is mostly found on epidermis and other species of *Propionibacterium spp*. are more common in endodontic infections. This result could be due to a sample contamination. Similar studies as well, report *Propionibacterium acnes* as the most common ones found[20,21]. Microbiological analysis shows the presence of *Prevotella melaninogenica*, *P. oralis* and *P. intermedia*. Pinheiro et al.[21] isolated *Prevotella spp.,* especially *P. intermedia* in the treated and obturated root canals and found connection to susceptibility to percussion.

Peptostreptococcus spp. are not found during the microbiological analysis. They are frequent during and after endodontic treatment as well as in retreatment cases. Peters at al.[20] found *Peptostreptococcus spp*. in more than 15% of the 58 tested root canals. In his research, Pinheiro[21] showed a strong connection between *Peptostreptococcus spp.,* isolated in more than 15% of the root canals and the occurance of a spontaneous pain of the endodontic treated teeth. Such a big difference between the results of this and similar other studies may be explained by difficult cultivation considering the sensitivity of this gram-positive anaerobe to oxygen.

Previous studies showed partial contradictory results about ozone efficacy on the endodontic pathogens. Nagayoshi et al.[10] tested the effect of ozonated water (4 μg/mL, 10 min) on *E. faecalis* incubated on dentine block for six days. They found a significant reduction, but not a total elimination of microorganisms.The study carried out by Hems et al.[11] does not show a significant reduction of *E. faecalis* biofilm using ozonated water, but proves a de-

crease in bacteria in the planktonic form. The study of Estrela[24] shows that none of the tested medicaments (ozonated water, ozone gas, 2.5% NaOCl, 2% chlorhexidine for 20 minutes) are effective on *E. faecalis* biofilm elimination. Huth et al.[25] tested the effect of ozone, ozonated water, 3% H_2O_2, 2.25% and 5.25% NaOCl and 2% chlorhexidine in different concentrations and exposure time of 1 minute on planktonic form of bacteria and biofilm. Their results showed that highly concentrated ozone gas and ozonated water are more effective in eliminating microorganisms in planktonic form. The effect of ozone (ozone-gas and ozonated water) in the same concentrations and exposure time is less effective on biofilm as compared to NaOCl or chlorhexidine solutions. *E. faecalis* biofilm was eliminated by highly concentrated ozone gas in a longer exposition time. These results could be explained by different study designs, different ozone concentrations and exposure time and planktonic and biofilm laboratory models. Lynch[26] mentioned redox reactions between ozone and reductants in brain-heart infusion rather than ozone and the bacterial strain. That may compromise results from Estrela's[24] study. Lynch criticised Hems's[11] study methodology and indicated that the experiment was biased by using extremely different dose of NaOCl and ozone.

The bacteria isolated from root canals are more resistant to alkaline stress in biofilm than in planktonic form which uses aggregation and extracellular transport of the specific proteins as a mechanism of survival[27].

Bacterial flora of the untreated canals shows the whole spectrum of gram-positive and gram-negative, mostly obligate anaerobic bacteria. In treated canals however, only several species are dominant, mostly gram-positive bacteria, facultative and obligate anaerobs equally[28,29]. The amount of bacterial species isolated from the treated root canals with chronic periapical lesions is large, but most studies confirm prevalence of enterococci and streptococci.

By releasing extracellular proteins and producing fimbriae, streptococci have the initiation capacity in biofilm forming[7], in which *E. faecalis* is often present. In this study *E. faecalis* was found in a small amount. The reason for that may be in the tooth selection. *E. faecalis* is mostly present in unsuccessful endodontic therapy and this study included, apart from endodontic retreatment therapy, untreated chronic apical periodontitis cases. Therefore, such a result could have been expected. Most findings of the treated root canals show mixed microflora. However, the results of some authors show a monocultural infection[21].

In monocultural infections, *E. faecalis* is the most common isolate, which is not typical in primary apical periodontitis[30,31]. In study of Sequeira[30] all samples taken from 22 root-filled teeth with persistent periradicular lesions were positive. *E. faecalis* was the most prevalent type detected in 77% of cases. These results were confirmed in the study by Gomes et al.[31] where *E. faecalis* was the most prevalent, detected in 77.8% of the cases. The reason for that is ecological tolerance and strong adaptive mechanism of surviving in hard conditions and high resistance of *E. faecalis* to calcium-hydroxide[32] and NaOCl[24].

In our study, after chemomechanical instrumentation, 14% of the samples were without aerobic and 53% without anaerobic bacteria, which is consistent with the findings of other authors.

Sjögren et al.[33] observed 50–60% negative samples after mechanical-chemical treatment and rinsing of the canals with 0.5% NaOCl. Peters et al.[34] found 76% bacteria-free canals after mechanical-chemical treatment and rinsing of the canals with 2% NaOCl. Chavez de Paz et al.[22] revealed that nonmutans streptococci, enterococci and lactobacilli mostly survive endodontic therapy of teeth with periapical lesions. Most frequent isolates are gram-positive bacteria (85%), *Lactobacillus spp*. (22%), nonmutans streptococci (18%) and *Enterococcus spp*. (12%) while gram-negative anaerobes are sporadic. Peters et al.[34] most frequently isolated *Prevotella intermedia*, *Capnocytophaga spp*., *Actinomyces odontolyticus*, *Propionibacterium acnes* and *Peptostreptococcus micros*. Similar results were obtained by Bebek et al.[35] also *Prevotella intermedia* and *Propionibacterium acnes* as the most frequently isolated species in infected root canals.

De Paz et al.[36] often found during the treatment *Lactobacillus spp*. and gram-positive cocci (*Streptococcus spp*., *Enterococcus spp*., coagulasa negative *Staphylococcus spp*. and *Peptostreptococcus spp*.). In the microbiological analysis of root canal of permanently obturated teeth with chronical periapical lesions Adib et al.[37] detected gram-positive facultative anaerobes as the most frequent (75%), staphylococci (19%), streptococci (17%), enterococci (8%) and *Actinomyces spp*. (8%), while obligate anaerobes take 17% and peptostreptococci 7%. Bacterial picture presented in their investigation coincides with findings of Molander et al.[28].

The results of this study show diversity of the remaining bacteria species in the root canal after chemomechanical instrumentation. Root canal ozone treatment statisticaly decreases bacterial count number as compared to the results after the chemomechanical instrumentation. Further studies should test the influence of higher concentration, longer exposure time, repeated ozone application and the possibility of using ozone in different vehiculum as intracanal medicament,but also as a treatment which has had a long term success.

Acknowledgements

This study was supported by Ministry of Science, Education and Sports, Grant No. 065-0352851-0410.

REFERENCES

1. SIQUEIRA JF JR, RÔÇAS IN, J Clin Microbiol, 43 (2005) 3314. DOI: 10.1128/JCM.43.7.3314-3319.2005. — 2. MUNSON MA, PITT-FORD T, CHONG B, WEIGHTMAN A, WADE WG, J Dent Res, 81 (2002) 761. DOI: 10.1177/154405910208101108. — 3. ESTRELA C, HOLLAND R, Appl Oral Sci, 11 (2003) 269. DOI: 10.1590/S1678-77572003000400002. — 4. SIQUEIRA JF JR, RÔÇHAS IN, FAVIERI A, LIMA KC, J Endod, 26 (2000) 331. DOI: 10.1097/00004770-200006000-00006. — 5. SIQUEIRA JF JR, MAGALHÃES KM, RÔÇHAS IN, J Enodod, 33 (2007) 667. DOI: 10.1016/j.joen.2007.01.004. — 6. DISTEL JW, HATTON JF, GILLESPIE MJ, J Endod, 28 (2002) 689. DOI: 10.1097/00004770-200210000-00003. — 7. CHÁVEZ DE PAZ L, Endod Topics, 9 (2004) 79. DOI: 10.1111/j.1601-1546.2004.00107.x. — 8. YAMAYOSHI T, TATSUMI N, Drugs Exp Clin Res, 19 (1993) 59. — 9. LYNCH E, Ozone: the revolution in dentistry. (Quintessence Publishing, Surrey, 2004). — 10. NAGAYOSHI M, KITAMURA C, FUKUIZUMI T, NISHIHARA T, TERASHITA M, J Endod, 30 (2004) 778. DOI: 10.1097/00004770-200411000-00007. — 11. HEMS RS, GULABIVALA K, NG YL, READY D, SPRATT DA, Int Endod J, 38 (2005) 22. DOI: 10.1111/j.1365-2591.2004.00891.x. — 12. SIQUEIRA JF JR, ROCAS IN, CARDOSO CC, MACEDO SB, LOPES HP, Rev Bras Odont, 57 (2000) 252. — 13. CARDOSO MG, DE OLIVEIRA LD, KOGA-ITO CY, JORGE AO, Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 105 (2008) 85. DOI: 10.1016/j.tripleo.2007.10.006. — 14. SILVEIRA AM, LOPES HP, SIQUEIRA JF JR, MACEDO SB, CONSOLARO A, Braz Dent J, 18 (2007) 299. DOI: 10.1590/S0103-644020070000400005. — 15. AL-KILANI MG, WHITWORTH JM, DUMMER PMH, Int Endod J, 36 (2003) 433. DOI: 10.1046/j1365-2591.2003.00675.x. — 16. SOARES JA, PIRES JÚNIOR DR, Braz Dent J, 17 (2006) 310. DOI: 10.1590/S0103-64402006000400009. — 17. MENEZES MM, VALERA MC, JORGE AOC, KOGA-ITO CY, CAMARGO CHR, MANCINI MNG, Int Endod J, 37 (2004) 311. DOI: 10.1111/j.0143-2885.2004.00799.x. — 18. TANDJUNG L, WALTIMO T, HAUSER I, HEIDE P, DECKER EM, WEIGER R, Int Endod J, 40 (2007) 845. DOI: 10.1111/j.1365-2591.2007.01279.x. — 19. VIANNA ME, GOMES BPFA, SENA NT, ZAIA AA, FERRAZ CCR, SOUZA FILHO FJ, Braz Dent J, 16 (2005) 175. DOI: 10.1590/S0103-6440 2005000300001. — 20. PETERS LB, WESSELINK PR, VAN WINKELHOFF AJ, Int Endod J, 35 (2002) 698. DOI: 10.1046/j.1365-2591.2002.00550.x. — 21. PINHEIRO ET, GOMES BPFA, FERRAZ CCR, SOUSA ELR, TEIXEIRA FB, SOUZA FILHO FJ, Int Endod J, 36 (2003) 1. DOI: 10.1046/j.1365-2591.2003.00603.x. — 22. CHAVEZ DE PAZ LE, DAHLÉN G, MOLANDER A, MÖLLER Å, BERGENHOLTZ G, Int Endod J, 36 (2003) 500. DOI: 10.1046/j.1365-2591.2003.00686.x. — 23. LAMBRIANIDIS T, KOSTI E, BOUTSIOUKIS C, MAZINIS M, Int Endod J, 39 (2006) 55. DOI: 10.1111/j.1365-2591.2005.01049.x. — 24. ESTRELA C, ESTRELA CRA, DECURCIO DA, HOLLANDA ACB, SILVA JA, Int Endod J, 40 (2007) 85. DOI: 10.1111/j.1365-2591.2006.01185.x. — 25. HUTH KC, QUIRLING M, MAIER S, KAMERECK K, ALKHAYER M, PASCHOS E, WELSCH U, MIETHKE T, BRAND K, HICKEL R, Int Endod J, 42 (2009) 3. DOI: 10.1111/j.1365-2591.2008.01460.x. — 26. LYNCH E, SWIFT EJ, J Esthet Restor Dent, 20 (2008) 287. DOI: 10.1111/j.1708-8240.2008.00195.x. — 27. CHÁVEZ DE PAZ LE, BERGENHOLTZ G, DAHLÉN G, SVENSÄTER G, Int Endod J, 40 (2007) 344. DOI: 10.1111/j.1365-2591.2006.01226.x. — 28. MOLANDER Å, REIT C, DAHLÉN G, KVIST T, Int Endod J, 31 (1998) 1. DOI: 10.1046/j.1365-2591.1998.t01-1-00111.x. — 29. SUNDQVIST G, FIGDOR D, PERSSON S, SJÖGREN U, Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 85 (1998) 86. DOI: 10.1016/S1079-2104(98)90404-8. — 30. SIQUEIRA JF JR, RÔÇHAS IN, Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 97 (2004) 85. DOI: 10.1016/S1079-2104(03)00353-6. — 31. GOMES BP, PINHEIRO ET, JACITO RC, ZAIA AA, FERRAZ CC, SOUZA-FILHO FJ, J Endod, 34 (2008) 537. DOI: 10.1016/j.joen.2008.01.016. — 32. PORTENIER I, WALTIMO T, ØRSTAVIK D, HAAPASALO M, J Endod, 31 (2005) 380. DOI: 10.1097/01.don.0000145421.84121.c8. — 33. SJÖGREN U, FIGDOR D, SPÅNGBERG L, SUNDQVIST G, Int Endod J, 24 (1991) 119. DOI: 10.1111/j.1365-2591.1991.tb00117.x. — 34. PETERS LB, VAN WINKELHOFF AJ, BUIJS JF, WESSELINK PR, Int Endod J, 35 (2002) 13. DOI: 10.1046/J.0143-2885.2001.00447.x. — 35. BEBEK B, BAGO G, ŠKALJAC G, PLEČKO V, MILETIĆ I, ANIĆ I, Coll Antropol, 33 (2009) 1159. — 36. CHAVEZ DE PAZ LE, MOLANDER A, DAHLÉN G, Int Endod J, 37 (2004) 579. DOI: 10.1111/j.1365-2591.2004.00845.x. — 37. ADIB V, SPRATT D, NG YL, GULABIVALA K, Int Endod J, 37 (2004) 542. DOI: 10.1111/j.1365-2591.2004.00840.x.

K. Prskalo

University of Zagreb, School of Dental Medicine, Department of Endodontics and Restorative Dentistry, Gundulićeva 5, 10000 Zagreb, Croatia
e-mail: prskalo@sfzg.hr

DJELOTVORNOST OZONA NA MIKROORGANIZME U KORIJENSKOM KANALU ZUBA

SAŽETAK

Svrha istraživanja bila je ispitati djelovanje ozona na bakterije preostale nakon kemijsko-mehaničke instrumentacije korijenskog kanala zuba. Istraživanje je provedeno na Zavodu za endodonciju i restorativnu stomatologiju Stomatološkog fakulteta Sveučilišta u Zagrebu. Sudjelovalo je 11 žena i 9 muškaraca upućenih na endodontsko liječenje. Dijagnozu neliječenog kroničnog apikalnog parodontitisa imalo je 17 zuba a kod 6 je bila potrebna revizija endodontskog liječenja. Ispitivani uzorak činila su 74 brisa iz 37 korijenskih kanala 23 zuba (10 sjekutića, 2 očnjaka, 8 pretkutnjaka, 3 kutnjaka). Prvi bris korijenskog kanala uzet je nakon završene kemijsko-mehaničke instrumentacije sterilnim papirnatim štapićem nakon ispiranja korijenskog kanala sterilnom fiziološkom otopinom. Posušeni kanal je tretiran ozonom 40 sekunda (HealOzone, Kavo, Njemačka). Nakon primjene ozona kanal je ispran sterilnom fiziološkom otopinom i uzet je drugi bris kanala. Brisovi su pohranjeni u transportne medije do kultivacije. Mikrobiološka analiza rađena je makromorfološkom, mikromorfološkom metodom i identifikacijom komercijalnim biokemijskim testovima. Analizom rezultata utvrđeno je statistički značajno smanjenje broja bakterija ($p<0,001$) nakon primjene ozona: ukupnog broja bakterija 82%, aerobnih 67%, anaerobnih 93%. Pojedinačnom analizom bakterija, značajno smanjenje nađeno je za Streptococcus mitis i Propionibacterium acnes ($p<0,05$). Ovo istraživanje je pokazalo djelotvornost ozona na smanjenje broja bakterija zaostalih nakon kemijsko-mehaničke instrumentacije korijenskog kanala.