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Antimikrobni učinak intrakanalne primjene ozona

Antimicrobial Effectiveness of Intracanal Ozone Treatment

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Sažetak

Svrha: Ovim se radom željela procijeniti količina aerobnih, anaerobnih i ukupnog broja bakterijskih kolonija nakon kemijsko-mehaničke obrade korijenskog kanala te dodatne obrade i dezinfekcije ozonom. **Materijali i metode:** U ovom istraživanju sudjelovala su dvadeset i tri pacijenta s periapikalnom lezijom (<5 mm) zabilježenom na retroalveolarnoj rendgenskoj slici i korišten je isti broj jednokorijenskih zuba (n=23). Za mikrobiološku analizu uzeta su tri brisa – neposredno prije (B1) mehaničke instrumentacije i irigacije 2,5-postotnom otopinom natrijeva hipoklorita i poslije toga postupka (B2) te nakon kemijsko-mehaničke obrade i dodatnog tretmana korijenskog kanala ozonom (B3). Brisevi su uzeti sterilnim papirnatim štapićima, bakterije su kultivirane, a rezultati su interpretirani nakon 14 dana koristeći se sustavom API 20A. Podaci su analizirani testom Wilcoxon Signed Rank. **Rezultati:** Razlike između skupina B1 i B2, B1 i B3, B2 i B3 za sve tri skupine bakterija: aerobne i anaerobne bakterije te ukupan broj bakterija bili su statistički značajni ($p<0,05$). **Zaključak:** Obje metode imale su dobar učinak i smanjile su broj bakterija prisutnih u korijenskom kanalu. Dodatnim tretmanom ozonom, nakon kemijsko-mehaničke obrade, još se smanjio broj aerobnih i anaerobnim bakterija, u usporedbi s mjerenjima neposredno prije kemijsko-mehaničke obrade i nakon nje. Kako bi se smanjio i reducirao broj bakterija, u svakodnevnoj se uporabi u kliničkoj praksi preporučuje dodatna primjena ozona kao dezinfekcijskog sredstva u korijenskom kanalu.

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Ključne riječi
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Uvod

Učinak mikroorganizama tijekom nastanka pulpnih i parodontnih bolesti odavno je poznat i dokumentiran. Važno je istaknuti da mikroorganizmi u korijenskom kanalu mogu rasti ne samo kao planktonske stanice ili u nakupinama (koagregati), nego mogu stvarati biofilmove koji se sastoje od složenih nakupina različitih mikroorganizama. Bakterije u korijenski kanal mogu dospjeti iz inficiranog pulpnog tkiva istog ili susjednog kanala i to su najčešće one koje su preživjele instrumentaciju i irigaciju tijekom endodontskog tretmana (1). Druga je mogućnost da su se naselile u korijenski kanal iz usne šupljine zbog neadekvatno osiguranog suhog radnog polja tijekom instrumentacije i irigacije, ili zbog nepotpune i neadekvatne posteendodontske opskrbe zuba koja ne osigurava hermetičko brtvljenje zbog koronarnog propuštanja (2,3). Velik je problem također to što nakon mehaničke i kemijske obrade korijenskih kanala i uporabe intrakanalnih medikamenata i uložaka između posjeta stomatologu, ostaju netretirana velika područja korijenskog kanala (4).

Primarnu infekciju korijenskih kanala uzrokuje mješovita flora s predominantnim gram-pozitivnim aerobima. Glavni uzročnici sekundarne infekcije su *Enterococus faecalis, Candida albicans* i *Actinomices israeli*. Da bi endodontski zahvat bio uspješan, moraju se potpuno ukloniti svi mikroorganizmi iz endodontskog prostora, sprječavajući tako primarnu i sekundarnu infekciju korijenskih kanala, što je vr-

Introduction

Influence of microorganisms on the formation of pulpal and periodontal disease is well known and documented. An important fact is that microbes in the root canals can grow not only as planctonic cells or in aggregates, co-aggregates, but they can also form biofilms consisting of a complex network of different microorganisms. Bacteria in the root canal may be derived from the infected pulp tissue of the same or adjacent canal that survived instrumentation and irrigation during endodontic treatment (1). Another possibility is that they are inhabited by microorganisms from the oral cavity due to inadequate control of dry working field during instrumentation and irrigation or because of the insufficient restorations made after endodontic treatment that do not provide a hermetic seal which occurs due to coronary failure (2, 3). Also, another major concern is that after using mechanical instrumentation and disinfecting solutions for irrigation or intracanal medicaments between appointments, large areas of the root canal system remained untouched (4).

The primary infection is caused by a mixed flora with predominant gram-positive anaerobic rod. The most important causes of secondary infection are *Enterococus faecalis, Candida albicans* and *Actinomices Israeli*. Successful endodontic therapy involves complete removal of all microorganisms from the endodontic space, preventing both primary and secondary infection of root canals, which is very difficult to achieve.

lo teško postići u cijelosti. Ishod endodontskog zahvata tako ovisi o vrsti i broju zaostalih mikroorganizama (5). Najčešće rabljeno sredstvo za dezinfekciju i irigaciju korijenskih kanala jest natrijev hipoklorit (NaOCl). On zbog svojih dezinfekcijskih svojstava i zato što otapa organske tvari, omogućuje kemijsku instrumentaciju korijenskog kanala s posebnim naglaskom na lateralne i akcesorne kanaliće koji se mehaničkom instrumentacijom ne mogu obraditi. Nedostatak toga sredstva je potencijalna toksičnost i velika površinska napetost koja smanjuje mogućnost ulaska u nepravilnosti kanala i dentinskih tubulusa (6). Smanjuje i elastičnost dentina te ga čini podložnijim frakturama (7). Istaknimo da se klorheksidin pokazao kao vrlo učinkovito sredstvo za dezinfekciju korijenskih kanala, a također bolje djeluje na bakteriju *Candida albicans* nego natrijev hipoklorit (8). Učinak 2-postotnog klorheksidina na *Enterococus faecalis* dokazan je u uvjetima *in vitro* i *in vivo* (9,10). Glavni mu je nedostatak to što loše uklanja organske tvari (11), što smanjuje njegovu sposobnost čišćenja i uklanjanja debrisa iz korijenskog kanala (12). Kalcijev hidroksid pokazao se kao vrlo učinkovito sredstvo za dezinfekciju endodontskog prostora između dvaju posjeta liječniku. Glavna mu je prednost, kao intrakanalnog medikamentnog uloška, visoka alkaličnost (pH 12) koju mikroorganizmi u endodontskom prostoru ne mogu preživjeti (12).

Iriganси se u korijenski kanal uglavnom unose špricom i iglom (13). Njihov potpuni kontakt sa zidom korijenskog kanala onemogućen je jer se glavnina irigansa usmjerava kroz vrh igle i to samo sredinom kanala, ali i zbog velike površinske napetosti otopine natrijeva hipoklorita (14,15). Zato su danas razvijene mnogobrojne tehnike irigacije korijenskih kanala, kao što je dezinfekcija foto-aktivacijom (16), aktivna irigacija kanala (17,18), primjena lasera u kanalima (19) i uređaji koji generiraju plazmu ili ozon (20, 21, 22).

Ozon (O_3) snažno je i učinkovito antimikrobno sredstvo. To je plavi plin koji ima tri vodikova atoma, nestabilan je i toksičan, iritira dišne puteve i vrlo je reaktivan. Snažan je oksidirajući agens (23) i rabi se za suzbijanje bakterija pri proizvodnji pitke vode (22). U mnogobrojnim studijama dobiveni su zanimljivi rezultati kada se ozonom tretirana voda koristila u stomatološkim radnim jedinicama (24). HealOzone (KaVo, Biberach, Njemačka) sustav je oslobađanja (generiranja) ozona iz atmosferskog kisika koji se sustavom cjevčica dovodi do sterilne silikonske kapice tj. na mjesto terapijske primjene. Ozon koji izlazi iz uređaja ima terapijsku vrijednost u koncentraciji od 4494 mg/m^3, odnosno 2100 ppm, a sterilna kapica, osim što stvara vakuum, služi kao barijera koja sprječava otpuštanje ozona u okolicu. Ozon se u toj jednokratnoj kapici rashlađuje brzinom od 615 cc/minuti, mijenjajući volumen plina unutar kapice više od 300 puta u svakoj sekundi. U usporedbi s ostalim, često primjenjivanim antisepticima, ozon ostvaruje jači učinak i istodobno potiče tkiva na cijeljenje i regeneraciju. Primjenjuje se u terapiji karijesnih lezija (25,26), u endodontskoj terapiji za redukciju mikrobne flore korijenskog kanala i ne djeluje negativno na periapikalna tkiva čime se smanjuje rizik od poslijeoperativnih komplikacija. Zbog njegovih baktericidnih, virucidnih i fungicidnih svojstava može se primjenjivati i u slučaju bolesti mekih tkiva usne šupljine (27,28).

The outcome of treatment depends on the type and number of residual microorganisms (5). The most commonly used root canal irrigation solution is sodium hypochlorite (NaOCl). As a disinfectant with the ability to dissolve organic substances it is used for canal instrumentation with particular emphasis on the lateral and accessory canals. A negative side-effect is the potential toxicity and high surface tension that reduces the possibility of entering the canal irregularities and dentinal tubules (6). It also reduces the elasticity of dentin and makes it susceptible to fractures (7). Chlorhexidine is also very effective for root canal disinfection and it has a better effect on *Candida albicans* than sodium hypochlorite (8). Effect of 2% chlorhexidine on *E. faecalis* has been demonstrated in *in vitro* and *in vivo* conditions (9, 10). The main dissadvantage is its poor organic structure removal (10), which reduces its ability to clean and remove debris from the root canal (11). Calcium hydroxide has proven to be very effective for disinfection of the endodontic space between the two visits. Its main advantage, as intracanal medicament dressing, is its high alkalinity (pH 12) at which microorganisms in endodontic space cannot survive (12). Irrigants have been traditionally applied by a syringe and needle (13). Adequate contact of the irrigant with the dentinal walls is enabled because most of the irrigant is potentiated only around the tip of the needle in combination with high surface tension of sodium hypochlorite (14, 15). That is why many various combinations of disinfecting solutions and irrigation devices such as photo-activated disinfection (16), active root canal irrigation (17, 18) and intracanal laser application (19) as well as ozone or plasma devices have been used (21, 21, 22).

Ozone (O_3) is an extremely powerful antimicrobial agent. It is a blue gas, which has three hydrogen atoms, it is unstable and toxic. It irritates the airways and is very reactive. It is a strong oxidizing agent (23) and is used to control bacteria in the production of drinking water (22). Numerous studies have shown interesting results when ozone treated water was used in dental work units (24). HealOzone (KaVo, Biberach, Germany) is a system of ozone generation from atmospheric oxygen which is then conducted through a sterile tube to the silicone cap to the area of therapeutic application. Ozone which leaves the device has a therapeutic value in a concentration of 4494 mg/m^3 or 2100 ppm. The sterile cap creates the vacuum and acts as a barrier to prevent the release of ozone into the environment. In the cap, ozone is cooled by speed of 615 cc/min, changing the volume of gas within the cap of more than 300 times every second. In comparison with other often applied antiseptics, ozone achieves higher performance while encouraging tissue to heal and regenerate. It is used for treatment of carious lesions (25, 26) and in endodontic therapy for reduction of microbial flora from the root canal without the negative properties on the periapical tissue, reducing the risk of postoperative complications. Its bactericidal, virucidal and fungicidal properties allow the application and treatment of the soft tissues diseases (27, 28).

The aim of this *in vivo* study was to compare the antibacterial action of intracanal ozone treatment, as additional disinfectant and the conventional chemomechanical endodontic treatment and to determine the reduction in the number

Svrha ove studije *in* vivo bila je usporediti antibakterijsku aktivnost intrakanalne primjene ozona kao dodatnog sredstva za dezinfekciju korijenskih kanala, s uobičajenom kemijsko-mehaničkom obradom te odrediti smanjenje broja aerobnih i anaerobnih bakterija te ukupnog broja bakterijskih kolonija. Nulta hipoteza bila je da nema razlike u antimikrobnom učinku između tih dvaju načina obrade korijenskih kanala.

Materijali i metode

Priprema uzoraka

Istraživanje je provedeno u Zavodu za endodonciju i restaurativnu stomatologiju Stomatološkog fakulteta Sveučilišta u Zagrebu. Sudjelovala su 23 ispitanika i za istraživanje je bilo potrebno isto toliko jednokorijenskih zuba (n=23). Prisutnost jednog korijenskog kanala dokazana je radiološkim snimkama u oba smjera – meziodistalnom i bukolingvalnom. U studiju su bili uključeni pacijenti koji su se Zavodu javili zbog endodontskog liječenja, zubi su im bili bez simptoma s negativnim nalazom na perkusiju, a na retroalveolarnoj rendgenskoj slici uočen je periapikalni proces ne veći od 5 milimetara. Svi ispitanici potpisali su informirani pristanak u kojem su bili objašnjeni ciljevi istraživanja, te poseban etički protokol koji je odobrilo Etičko povjerenstvo Stomatološkog fakulteta u Zagrebu.

Za mikrobiološku analizu uzeta su tri brisa korijenskog kanala – neposredno prije (B1) tretmana, nakon mehaničke obrade i irigacije 2,5-postotnim natrijevim hipokloritom (B2) i nakon kemijsko-mehaničke obrade i naknadne intrakanalne primjene ozona (B3). Najprije je, nakon što je postavljen gumeni štitnik (Dental Dam, Roeko, Langenau, Njemačka), izrađen pristupni trepanacijski otvor, uklonjen je koronarni dio pulpe i kavitet je ispran jednim mililitrom fiziološke otopine (Pliva, Zagreb, Hrvatska). Nakon toga izmjerena je radna duljina apeks-lokatorom (ES-02, Artronic, Zagreb, Hrvatska) i proširivačem # 15 (Dentsply-Maillefer, Ballaigues, Švicarska). Tako je napravljen prostor za sterilni papirnati štapić # 15 (Dentsply-Maillefer, Ballaigues, Švicarska) kojim je uzet prvi bris korijenskog kanala prije početka instrumentacije (B1). Nakon uzimanja brisa korijenski kanali ručno su instrumentirani proširivačima i strugačima *step back* tehnikom do veličine # 25, a poslije toga je obavljena dodatna instrumentacija strojnom tehnikom EndoEZE (Ultradent Products, South Jordan, SAD) do veličine # 40. Svaki kanal ispirao se 30 sekundi s 2 ml 2,5-postotne otopine natrijeva hipoklorita između svakog idućeg instrumenta jednokratnom špricom i iglom 30 G (BD Microlance, Becton Dickinson, Madrid, Španjolska). Završno ispiranje obavljeno je s 5 ml fiziološke otopine i jednokratnom špricom od 5 ml i iglom 30 G. Prije uzimanja brisa korijenski kanal je navlažen sterilnom fiziološkom otopinom i dodatno ostrugan pilicom Hedström #25 (Dentsply-Maillefer, Ballaigues, Švicarska) kako bi se mobilizirale bakterije na stijenki kanala. Mikrobiološki bris endodontskog prostora uzet je na kraju instrumentacije sterilnim papirnatim štapićem # 25 (B2). Nakon kemijsko-mehaničke obrade proveden je 40-sekundni tretman HealOzonom (Kavo, Biberach, Njemačka) u

of aerobic, anaerobic, and total number of bacterial colonies. The null hypothesis was that there were no differences between antimicrobial efficacies of these two root canal disinfection techniques.

Materials and methods

Preparation of specimens

The study was carried out at the Department of Endodontics and Restorative Dentistry, School of Dental Medicine, University of Zagreb, Croatia. Twenty three patients with the same number of single rooted teeth (n=23) were involved in the study. The presence of a single canal was determined by radiographs taken in both mesiodistal and buccolingual directions. The patients were referred to the Department because of the need for endodontic treatment. Inclusion criteria were: single rooted teeth without any symptoms, negative percussion test and periapical processes up to 5 mm observed on retroalveolar radiogram or orthopantomogram. The institutional Ethics Committee has approved the study, and patients had given their informed consent to their participation in the study.

For the microbiological analysis three swabs were taken: immediately before the treatment (B1), after mechanical instrumentation and irrigation with 2.5% sodium hypochlorite (B2) and after chemomechanical treatment and additional intracanal ozone application (B3). In the first procedure after placing a rubber guard (Dental Dam, Roeko, Langenau, Germany), an access cavity was created, the coronal portion of the pulp was removed and the cavity was rinsed with 1 mL of saline solution (Pliva, Zagreb, Croatia). The working length was measured using apex locator (ES-02, Artronic, Zagreb, Croatia) and a K- file # 15 (Dentsply Maillefer, Tulsa, Oklahoma, USA). The root canal swab was taken with a sterile paper point # 15 (Dentsply Maillefer, Tulsa, Oklahoma, USA) before the instrumentation (B1). After the root canal swab was taken, the chemomechanical treatment was performed. The root canals were manually instrumented with K files and K reamers using the *step back* technique to size # 25, and subsequently conducted additional mechanical instrumentation technique EndoEZE (Ultradent Products, South Jordan, USA) to the size # 40. Each canal was irrigated with 2 mL of 2.5% NaOCl for 30 s between each instrument using a disposable 2 mL syringe and 30-gauge needle (BD Microlance, Becton Dickinson, Madrid, Spain) and were finally rinsed with 5 ml of saline solution using the disposable 5 ml syringe and 30-G needle. Before swab taking, root canals were moistened with sterile saline and ground with H file # 25 (Dentsply Maillefer, Tulsa, Oklahoma, USA) for further bacterial mobilization from the canal walls. Microbiological swab of endodontic space at the end of instrumentation was taken with sterile paper point # 25 (B2). After the chemomechanical canal treatment, HealOzone (Kavo, Biberach, Germany) treatment was performed for 40 seconds in the EN-

ENDO-modu, s endodontskim nastavkom postavljenim u korijenski kanal i silikonskom kapicom koja je služila da se dobije vakuum u endodontskom prostoru. Nakon tretmana HealOzonom uzet je mikrobiološki bris (B3) endodonstkog prostora sterilnim papirnatim štapićem, no prije ponovljen je postupak struganja stijenki kanala sterilnom pilicom Hedström #25. Zatim su korijenski kanali napunjeni štapićima gutaperke (Dentsply-Maillefer, Ballaigues, Švicarska) i punilom AH Plus (Dentsply De-Trey, Konstanz, Njemačka) tehnikom hladne lateralne kondenzacije. Nakon završenog tretmana napravljene su kontrolne retroalveolarne rendgenske snimke punjenja.

Papirnati su štapići, odmah nakon uzimanja brisa, pohranjeni u transportni medij za anaerobe WMGA i tijekom 30 minuta dostavljeni mikrobiološkom laboratoriju. Ondje su nasađeni na podlogu Collumbia agar, Zambon, Hunt, Mandel i Corman hranilišta za anaerobe te na krvni agar za aerobe. Antimikrobni učinak korištenih metoda potvrđen je nakon 14 dana kada su izmjerene kulture bakterija, a rezultati su očitani s pomoću sustava API 20A.

Statistička analiza

Podaci su analizirani mjerenjem broja bakterija prije zahvata i nakon njih. Izračunate su srednje vrijednosti i standardna devijacija za broj bakterijskih kolonija nakon mehaničke instrumentacije korijenskog kanala uz irigaciju 2,5-postotnim natrijevim hipokloritom i redukcije bakterija poslije dodatnog intrakanalnog tretiranja korijenskog kanala ozonom za svaku skupinu bakterija posebno (aerobi, anaerobi i ukupan broj). Budući da podaci nisu bili normalno distribuirani, usporedbu srednjih vrijednosti obavljena je neparametrijskim testom Wilcoxon Signed Rank uz razinu značajnosti 0,05, za što smo se koristili programom SAS 8,2 (SAS Institute Inc, Sjeverna Karolina, SAD).

Rezultati

Vrijednosti dobivene mjerenjem broja bakterijskih kolonija poslije mehaničke instrumentacije korijenskog kanala uz irigaciju 2,5-postotnim natrijevim hipokloritom i broja bakterijskih kolonija nakon dodatnog intrakanalnog tretiranja korijenskog kanala ozonom, nalaze se u tablicama i grafikonima. U tablici 1. je distribucija rezultata (aritmetička sredina, medijan, standardna devijacija i koeficijent varijacije) prije tretmana korijenskih kanala i nakon toga postupka. Slika 1. prikazuje rezultate dobivene nakon analize broja aerobnih i anaerobnih te ukupnog broja bakterijskih kolonija uzetih

DO mode, with endodontic extension installed into the root canal and a silicon cap which was used to achieve a vacuum in the endodontic space. Before the third swab taking, root canals were moistened with sterile saline and ground with H file # 25 (B3). The root canals were then obturated with guttapercha points (Dentsply Maillefer, Tulsa, Oklahoma, USA) and AH Plus (Dentsply De-Trey, Konstanz, Germany) root canal sealer using cold lateral condensation technique. Control retroalveolar radiograms were made at the end of treatment.

Immediately after taking the swabs, paper points were stored in WMGA transport medium for anaerobes and transported to the microbiology laboratory within 30 minutes. In the microbiologic laboratory they were inoculated onto Collumbia agar base, Zambon, Hunt, Mandel and Corman base for anaerobes, and Blood agar for aerobes. The antimicrobial efficacy of the used methods was confirmed by the culture method and the results were interpreted after 14 days using the API 20A system.

Data analysis

Data were analyzed by measuring the number of bacterial colonies before and after different intracanal treatments. Mean values and standard deviations for the number of bacterial colonies after mechanical instrumentation and irrigation with 2.5% sodium hypochlorite and the reduction of bacteria after additional root canal treatment with ozone for each particular group of bacteria (aerobes, anaerobes and total number) were calculated. Data were not normally distributed and for comparison of mean values non-parametric Wilcoxon Signed Rank Test with the significance level of 0.05 was used. Results were presented graphically (Box plot and histogram). For data analysis, SAS 8.2 (SAS Institute Inc, North Carolina, USA) was used.

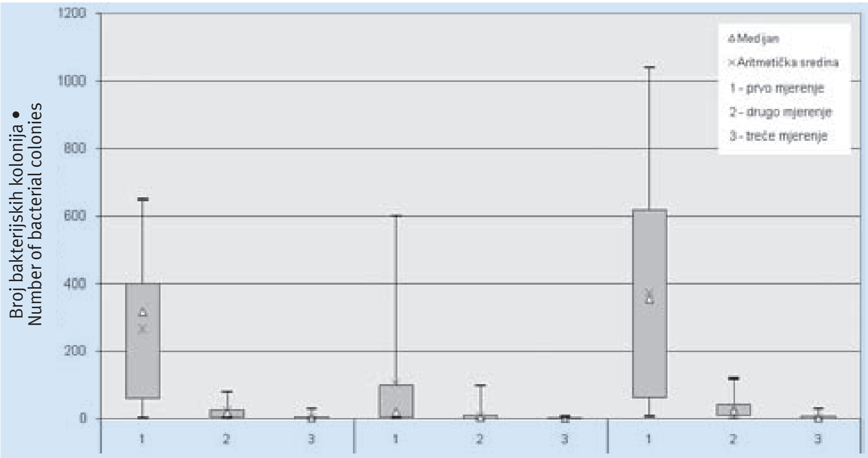
Results

Values obtained by measuring the number of bacterial colonies after mechanical instrumentation and root canal irrigation with 2.5% sodium hypochlorite and the number of bacterial colonies after additional intracanal ozone treatment are presented in tables and figures. Table 1 presents the distribution of the results (mean, median, standard deviation and coefficient of variation) before and after treatment protocols. Figure 1 presents the results obtained after analyzing the number of aerobic, anaerobic and total bacterial colonies of each swab. Figure 2 present the reduction of aerobic bacte-

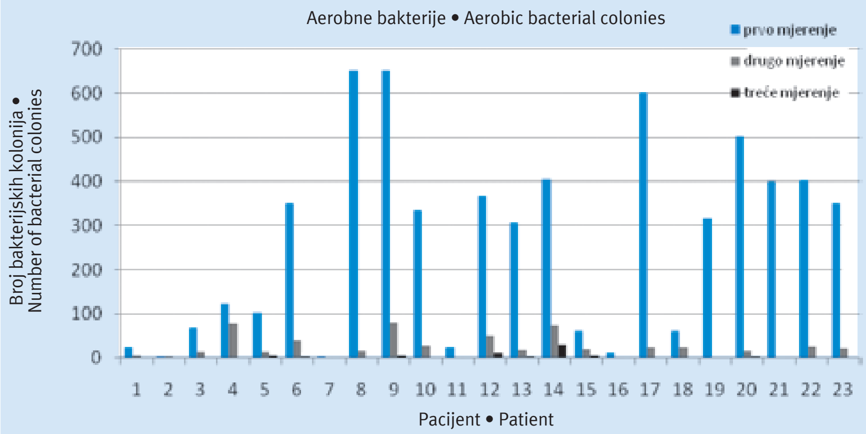
Tablica 1. Aritmetička sredina, standardna devijacija, koeficijent varijacije i medijan za aerobne i anaerobne bakterije te ukupan broj bakterijskih kolonija. Zbog velikog koeficijenta varijacije (> 30%) medijani su prikazani kao reprezentativne srednje vrijednosti.
Table 1 Arithmetic mean, standard deviation, coefficient of variation and median for aerobic, anaerobic and total number of bacterial colonies. Because of the great coefficient of variation (> 30%) we showed medians as representative mean value.

	Aerobne bakterije				Anaerobne bakterije				Ukupno bakterije			
	arit. sredina	STD	CV*	medijan	arit. sredina	STD	CV*	medijan	arit. sredina	STD	CV*	medijan
1. mjerenje	265,9	217,555	81,8	316	105,8	170,801	161,5	20	373,4	310,295	83,1	355
2. mjerenje	24,4	24,834	101,8	18	9,1	20,552	225,1	3	33,5	32,903	98,2	24
3. mjerenje	3,7	6,379	174,7	2	1,0	1,894	181,6	0	4,7	6,691	142,5	2

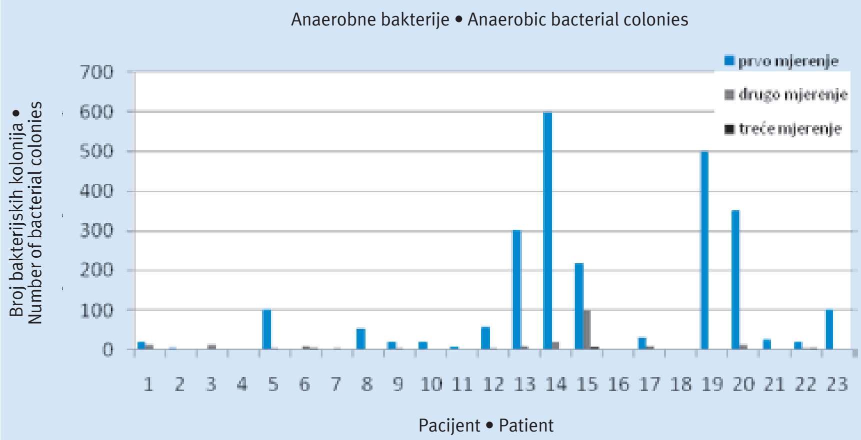
* CV – koeficijent varijacije • Coefficient of variation



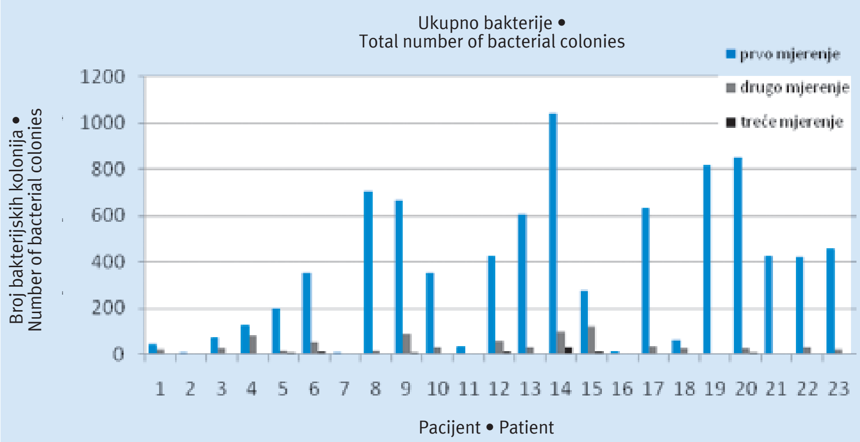
Slika 1. Usporedba aerobnih i anaerobnih bakterija te ukupnog broja bakterijskih kolonija.
Figure 1 Comparison of aerobic, anaerobic and total number of bacterial colonies



Slika 2. Broj aerobnih bakterijskih kolonija neposredno prije i nakon mehaničke obrade te irigacije 2,5-postotnim natrijevim hipokloritom i poslije dodatne obrade korijenskog kanala HealOzonom.
Figure 2 Number of aerobic bacterial colonies immediately before the treatment, after chemomechanical instrumentation and irrigation with 2.5% sodium hypochlorite and after additional HealOzone root canal treatment.



Slika 3. Broj anaerobnih bakterijskih kolonija neposredno prije i nakon mehaničke obrade te irigacije 2,5-postotnim natrijevim hipokloritom i poslije dodatne obrade korijenskog kanala HealOzonom.
Figure 3 Number of anaerobic bacterial colonies immediately before the treatment, after chemomechanical instrumentation and irrigation with 2.5% sodium hypochlorite and after additional HealOzone root canal treatment.



Slika 4. Ukupan broj bakterijskih kolonija neposredno prije i nakon mehaničke obrade te irigacije 2,5-postotnim natrijevim hipokloritom i poslije dodatne obrade korijenskog kanala HealOzonom.
Figure 4 Total number of bacterial colonies immediately before the treatment, after chemomechanical instrumentation and irrigation with 2.5% sodium hypochlorite and after additional HealOzone root canal treatment.

nakon svakog brisa. Na slici 2. su vrijednosti u redukciji broja aerobnih bakterija, slika 3. prikazuje vrijednost u redukciji broja anaerobnih bakterija, a slika 4. vrijednosti u redukciji ukupnog broja bakterija nakon mehaničko-kemijske obrade korijenskog kanala i broja bakterija nakon dodatnog intrakanalnog tretiranja korijenskog kanala HealOzonom.

Statistički značajna razlika zabilježena u broju aerobnih bakterijskih kolonija između skupina B1 i B2 (p<0,0001), B2 i B3 (p<0,0001), B1 i B3 (p<0,0001). Isti princip uporabljen je i za analizu razlika skupina i parova skupina kod anaerobnih kolonija. Statistički značajna razlika zabilježena je između skupina B1 i B2 (p<0,0001), B2 i B3 (p<0,0001) i B1 i B3 (p=0,0002). Statistički značajna razlika zabilježena je i u ukupnom broju bakterijskih kolonija između skupina B1 i B2 (p<0,0001), B2 i B3 (p<0,0001) i B1 i B3 (p=0,0002). Razlike između aerobnih i anaerobnih bakterija te ukupnog broja bakterijskih kolonija analizirane su i uspoređene s nultom hipotezom u kojoj ne postoji razlika između početnih mjerenja i nakon kemijsko-mehaničke obrade korijenskog kanala te poslije dodatne obrade korijenskog kanala ozonom. Korišten je Friedmanov test i uočene su statistički značajne razlike u distribuciji između sva tri mjerenja (p<0,0001).

Rasprava

Mikrobiološka flora korijenskog kanala najvažniji je čimbenik o kojem ovisi uspjeh endodontskog tretmana. Poznato je da bakterije i njihovi bioprodukti imaju glavnu ulogu u patogenezi periapikalnog parodontitisa (29). Sekundarne infekcije korijenskog kanala nastaju kao posljedica neuspješnog endodonstskog liječenja zbog perzistiranja bakterija u endodontskom prostoru. Zato endodontski zahvat treba biti ograničen na samo nekoliko posjeta stomatologu, a on se pritom treba koristiti protokolima kojima se nastoji postići uspješna i učinkovita dezinfekcija korijenskih kanala. Ovo istraživanje provedeno je kako bi se ustanovilo može li dodatni tretman korijenskog kanala ozonom smanjiti broj zaostalih bakterija, pa tako i sekundarne infekcije. Nulta hipoteza – da nema razlike između dvaju različitih antimikrobnih tretmana korijenskih kanala, odbačena je. Zaključeno je da dodatni tretman, tj. dezinfekcija korijenskih kanala ozonom, može smanjiti broj aerobnih i anaerobnih bakterija te ukupan broj bakterijskih kolonija koje ostaju u korijenskim kanalima nakon klasične kemijsko-mehaničke obrade.

Objavljena su mnoga istraživanja temeljena na tehnikama dezinfekcije korijenskih kanala, ali često s kontradiktornim rezultatima. U ovoj studiji *in vivo* proučavao se antimikrobni učinak intrakanalne primjene ozona koji se može rabiti kao nadopuna klasičnoj kemijsko-mehaničkoj obradi kanala. Rezultati jasno pokazuju prednosti dodatnog korištenja ozona u odnosu na kemijsku i mehaničku obradu korijenskih kanala i mogu se usporediti s mnogim studijama koje su dokazale snažan antimikrobni učinak ozona korištenog u korijenskom kanalu (30, 31, 32, 29), pri čemu se najbolji učinak postiže kada se ozon uporablja u kanalu s minimalno zaostalog organskog debrisa (33). Sredstva za dezinfekciju,

rial colonies, Figure 3 shows the reduction of anaerobic bacterial colonies and Figure 4 present the reduction of the total number of bacterial colonies after chemomechanical root canal treatment and the number of bacterial colonies after additional intracanal HealOzone treatment.

A statistically significant difference was found in the number of aerobic bacterial colonies between groups B1 and B2 (p<0.0001), B2 and B3 (p<0.0001) and B1 and B3 (p<0.0001). The same principle was used to analyze the differences between groups and group pairs in anaerobic bacteria group. A statistically significant difference was found between groups B1 and B2 (p<0.0001), B2 and B3 (p<0.0001) and B1 and B3 (p=0.0002). A statistically significant difference was found between the total number of bacterial colonies in groups B1 and B2 (p<0.0001), B2 and B3 (p<0.0001) and B1 and B3 (p=0.0002). The differences between the number of aerobic bacterial colonies, number of anaerobic bacterial colonies and total number of bacterial colonies were analyzed with reference to null-hypothesis in which there are no differences between the initial measurements and the chemomechanical root canal treatment and after additional intracanal ozone application. Data were analyzed by Friedman Test and a statistically significant difference was found between these three measurements (p<0.0001).

Discussion

Microbial flora of the root canal is the most important factor influencing the success of endodontic treatment. It is known that bacteria and their byproducts play a major role in the pathogenesis of periapical periodontitis (29). The persistence of bacteria in endodontic space results in a failed endodontic treatment and secondary infection. Endodontic treatment has to be reduced to as few visits as possible using protocols that can achieve root canal disinfection effectively. This study was aimed to determine if the additional treatment of root canals with ozone can further diminish the number of residual bacteria and reduce the occurence of the secondary infection. The null-hypothesis that there were no differences between antimicrobial efficacies of two root canal disinfection techniques used in this study was rejected. It was determined that additional intracanal ozone treatment significantly reduced the aerobic, anaerobic and total number of bacterial colonies.

Many studies on root canal disinfection techniques have been published but most of them with contradictory results. This *in vivo* study evaluated the antimicrobial effect of intracanal ozone treatment which can be used as adjunct to chemomechanical canal preparation. The results clearly showed the superiority of additional ozone treatment in comparison to chemomechanical preparation and can be compared and confirmed by the numerous studies, which have proven a powerful antimicrobial effect of ozone applied in the root canal (30, 31, 32, 29) with the best performance achieved by the use of ozone in the root canal in which there is minimal smear organic debris (33). Disinfection agents such as NaOCl require direct contact with the bacteria which is often impossible in peripheral areas of the root canal such as anaskao što je NaOCl, zahtijevaju direktan kontakt s bakterijama, što je ponekad nemoguće, posebice u perifernim dijelovima korijenskih kanala, kao što su anastomoze i lateralni kanali ili u završnom apikalnom dijelu kanala (12). Pritom konačna obrada kanala ozonsko-ultrazvučnim instrumentima ili kombinacijom ozonirane vode ili plina ozona u korijenskom kanalu može pomoći u dodatnom uklanjanju bakterija. HealOzone (KaVo, Biberach, Njemačka) zatvoreni je sustav generiranja ozona iz atmosferskog kisika koji se sustavom cjevčica dovodi do sterilne silikonske kapice tj. na mjesto terapijske primjene. Na taj način ozon je usmjeren samo na mjesto djelovanja, a zanemariv je štetan rasap po ustima i radnom prostoru (25), što mu daje prednost u odnosu na ostale sustave. Ozon je također vrlo učinkovito sredstvo za dezinfekciju površina. Zbog spontanog i kataliziranog raspada molekule prikladan je i za korištenje u ustima tijekom kirurških zahvata te tretmana karijesa i korijenskih kanala. Tretman od 40 sekundi, primijenjen u ovome istraživanju, odabran je nakon što ga je proizvođač preporučio za završnu obradu i dezinfekciju korijenskih kanala. Nedostatak pri korištenju HealOzona u području endodoncije jest teško postizanje potrebnog vakuuma, posebice kod malih i destruiranih kruna zuba, što ograničava njegovu uporabu na tom polju.

U ovom istraživanju promatrana je redukcija u broju aerobnih i anerobnih bakterija i ukupnog broja bakterijskih kolonija, pa ga je teško usporediti s ostalim, u literaturi dostupnim studijama, u kojima se jasno iznose promjene u broju pojedinih sojeva bakterija. Od ukupnih bakterijskih kolonija nađenih u korijenskom kanalu prije instrumentacije, aerobne bakterije činile su 84 posto, a anaerobne 16 posto, što je u skladu s istraživanjem Pinheira i njegovih suradnika. Oni su u svojoj studiji provedenoj na 30 punjenih korijenskih kanala dokazali predominaciju gram-pozitivnih aeroba i fakultativnih anaeroba među kojima su dominirali *E. faecalis, Streptococcus, Peptostreptococcus* i *Actinomyces* (34). Peciuliene i kolege izolirali su mikroorganizme iz sekundarno inficiranog endodontskog prostora i pronašli *E. faecalis* u 21 od 33 izolata, a kod 6 su pronađene kvasnice, osobito *Candida albicans* (35). Visokoj virulenciji *E. fecalis* vjerojatno pridonosi sposobnost invazije u dentinske tubule i adherencija na kolagen u ljudskom serumu. Prema mišljenju Molandera i suradnika, fakultativni anaerobi, naročito G+, većinska su mikroflora u slučaju sekundarnih endodontskih infekcija jer mogu preživjeti u takvim uvjetima uz nisku metaboličku aktivnost, primjerice, koronarnim mikropropuštanjem kroz punjenje mijenjaju metaboličku aktivnost na višu razinu te se tako umnožavaju (36). Nakon mehaničke obrade i irigacije 2,5-postotnom otopinom natrijeva hipoklorita značajno je smanjen broj aerobnih i anerobnih bakterija u odnosu na početno mjerenje prije mehaničko-kemijske obrade kanala. To je u skladu s istraživanjima Siqueire i njegovih kolega koji su se koristili 2,5-postotnim NaOCl-om tijekom tretmana nekrotične pulpe i apikalnog parodontitisa (37), a za veće koncentracije NaOCl-a poznato je da imaju i veći iritacijski učinak na stanice apeksa i periapikalnog tkiva (38). Silveira i suradnici dokazali su da otopina 2,5-postotnog NaOCl-a i 2-postotnog klorheksidina potpuno uklanja bakterijsku infekciju s *E. faecalis* nakon kontakta od 30 sekundi (38).

tomoses, lateral canals or the most apical part of the main root canal (12) and final irrigation using ozone ultrasonic instruments or a combination of ozone water and ozone gas leakage through the root canal can help reducing the bacteria. HealOzone (KaVo, Germany) is a system of ozone generation where ozone is directed only at the place of action, while the harmful ozone influence in the mouth and working space is negligible (25). This gives it an advantage over other ozone generating systems. Ozone gas is a highly effective surface disinfectant. As a result of the spontaneous and catalyzed breakdown of the molecule, it is suitable for use in the mouth during surgical interventions as well as caries or root canal treatment. The time period of 40 s of disinfection used in this study was chosen according to the time recommended by the manufacturer for the final root canal disinfection protocol. The disadvantage of using HealOzone in the field of endodontics is the difficulty of achieving the required vacuum especially for small and destroyed tooth crowns, which limits its use in this field.

Most of the studies in the available literature dealt with the change in the number of individual bacterial strains, whereas the present study was focused on the number of aerobic, anerobic and the total number of bacterial colonies, and as such, the study was difficult to compare with others. Aerobic bacteria accounted for 84% of the total bacterial colonies found in the root canal before instrumentation and anaerobic bacteria for the remaining 16%, which is consistent with research of Pinheiro et al. in which 30 filled root canals showed predomination of aerobic gram-positive and facultative anaerobes dominated by *E. faecalis*, *Streptococcus, Fusobacterium* and *Actinomyces* (34). Peciuliene et al. isolated microorganisms from secondary infected endodontic area and found the presence of *E. faecalis* in 21 of 33 isolates, while in 6 isolates they found the presence of yeast, especially *Candida albicans* (35). High virulence of *E. faecalis* likely contributes to the ability of invasion into dentinal tubules and adherence to the collagen in the presence of human serum. According to Molander et al., facultative anaerobes, particularly G +, make the majority of secondary microflora in endodontic infections because they can survive in conditions with low metabolic activity, such as coronary leakage; they change its metabolic activity to a higher level and reproduce (36). The mechanical treatment and irrigation with 2.5% sodium hypochlorite significantly reduced the number of aerobic and anaerobic bacteria, when compared to the initial measurement before chemomechanical treatment. This result is consistent with the research of Siqueira et al. who used 2.5% NaOCl in the treatment of necrotic pulp and apical periodontitis (37). It is known that higher concentrations of NaOCl possess a greater irritation effect on the apical cells and periapical tissues (38). Silveira et al. have shown that 2.5% NaOCl solution and 2% chlorhexidine completely remove bacterial infection of E. faecalis after 30 s of contact (39).

In the present study, application of ozone gas in combination with chemomechanical root canal treatment has led to a significant reduction of bacteria which makes it potentially usable and good as additional root canal treatment

U našem istraživanju, primjenom ozona u kombinaciji s kemijsko-mehaničkom obradom korijenskih kanala, dodatno je smanjen broj aerobnih i anaerobnih bakterijskih kolonija, što ga čini potencijalno korisnim dodatnim tretmanom pri obradi korijenskih kanala. Nagayoshi i suradnici dokazali su da ozonirana voda ima gotovo jednako antimikrobno djelovanje kao i 25-postotni NaOCl, posebice u kombinaciji s ultrazvučnom tehnikom obrade kanala, a pritom je zabilježena niska toksičnost na stanice u kulturama. Oznonirana voda može se smatrati potencijalnim sredstvom za dezinfekciju korijenskih kanala jer je manje toksična od NaOCl-a koji može dovesti do nekroze, a ozonirana je voda iznimno biokompatibilna (29). Zato su Steier i Steier predložili kombinaciju manje toksičnog NaOCl-a (1,25 %) i ozona (40). Rezultate Nagayoshija i suradnika potvrdili su Huth i suradnici – broj bakterija iz korijenskih kanala (biofilm koji se stvorio nakon izlaganja zuba *Pseudomonas aeruginosi*, *Enterococcus faecalis*, *Peptostreptococcus micros* i *Candidi albicans*) smanjio se nakon primjene plina ozona i/ili ozonirane vode, pri čemu se rabio poseban uređaj za izravnu aplikaciju ozona u korijenski kanal, točno na mjesto primjene tj. na inficirane zidove korijenskih kanala (41, 42). Vitrej i suradnici usporedili su učinkovitost četiriju sustava –HealOzona, 3-postotnog NaOCl-a, MTAD-a (Dentsplay, Tulsa, SAD) i sistema Endox Endodontics (Endox S.r.l., Carugo, Italija) kao sredstava za irigaciju korijenskih kanala. Učinkovitost 3-postotnog NaOCl-a, MTAD-a i HealOzona bila je podjednaka. Sustav Endox pokazao je najmanju antibakterijsku učinkovitost. Zaključeno je da ozon ima veliki potencijal za uporabu u endodontskoj terapiji te da u znatnoj mjeri reducira bakterijske infekcije u sustavima korijenskih kanala (33). Stoll i suradnici dokazali su pozitivan učinak ozona (120 sekundi, HealOzone generator, KaVo, Biberach, Njemačka) na *E. faecalis* u usporedbi s konvencionalnim irigansima – 3-postotnim vodikovim peroksidom, 0,2-postotnom otopinom klorheksidina, 1,5-postotnim i 3-postotnim natrijevim hipokloritom (43). Ozon je potencijalni oksidans sa sposobnošću stupanja u redoks-reakcije. Zato se preporučuje ozon primjenjivati pod tlakom, kako bi mogao penetrirati kroz korijenski kanal i djelovati irigacijski. U uvjetima *in vivo* korijenski kanal sadržava biofilm, čiji sastojci, poput željeza, pospješuju antimikrobnu aktivnost ozona i oslobađanje snažnih hidroksilnih radikala. Uporabom uljnih preparata ozona, u usporedbi s intrakanalnim medikamentnim sredstvima kao što je kalcij hidroksid, ostvaruje se učinkovitije djelovanje na bakterije u kanalu i odlična biokompatibilnost s periradikularnim tkivima (42). Lynch i Swift zaključili su da je ozon najsnažnije antimikrobno oksidirajuće sredstvo kojim se stručnjaci mogu koristiti u endodonciji i da je vodena otopina ozona visoko biokompatibilna u odnosu na ostale korištene iriganse. Ozon je potrebno primijeniti kako bi se omogućio što bolji učinak uklanjanja mikroorganizama iz korijenskih kanala (44). Vodena otopina ozona također je mnogo biokompatibilnija i manje toksična od natrijeva hipoklorita. Primjena plina ozona na karijesne lezije (s pečaćenjem ili bez njega), ili u inficirane korijenske kanale rabi se i u kliničkoj praksi. Poznat je i učinak ozona na liječenje parodontitisa, periimpantitisa i endodontskih infekcija, ali potrebna su dodatna

method. Nagayoshi et al. have shown that ozone water has almost the same antimicrobial activity as 2.5% NaOCl, especially in combination with ultrasonic canal treatment, with low cellular toxicity. Ozone water can be considered to be a potential root canal disinfectant and is less cytotoxic than NaOCl which can cause necrosis while ozone water is exceptionally biocompatible (29). Steier and Steier have suggested combining a less cytotoxic concentration of NaOCl (1.25%) with ozone (40). The results of Nagayoshi et al. have been confirmed by Huth et al. in a study where the number of bacteria from the root canal (biofilm formation after incubation of teeth with *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Peptostreptococcus micros* and *Candida albicans*) was reduced using ozone gas and/or ozone water. Special devices used for ozone allowed the direct application of ozone directly into the root canal and resulted in a targeted local action on the infected root canal walls (41, 42). Vitrej et al. compared the effectiveness of four systems: Heal Ozone, 3% NaOCl, MTAD (Dentsplay Tulsa, USA) and Endox Endodontics System (Endox S.r.l., Carugo, Italy) as a tool for root canal irrigation. The effectiveness of 3% NaOCl, MTAD and HealOzone was similar. Endox system showed the least antibacterial efficacy. It was concluded that ozone has a great potential for use in endodontic therapy and it significantly reduces bacterial infection in the root canal system in addition to the standard chemomechanical cleaning (33). Stoll et al. have shown positive effects of ozone (120 seconds, HealOzone generator, KaVo) in *E. faecalis* compared with conventional irrigants: 3% hydrogen peroxide, 0.2% chlorhexidine solution, 1.5% and 3% sodium hypochlorite (43). Ozone is a potent oxidant that has the capability of entering into redox reaction. The recommendation is therefore to use ozone under pressure, so it can penetrate through the root canal and cause the effect of irrigation. *In vivo,* root canal contains biofilm, the ingredients of which, such as iron, enhance the antimicrobial activity of ozone and the release of powerful hydroxyl radicals. The use of ozone oil products compared with intracanal medications such as calcium hydroxide, achieves effective action of the bacteria in the canal with excellent biocompatibility with the periradicular tissues (42). Lynch and Swift concluded that ozone is the most powerful antimicrobial and oxidant we can use in endodontics, and that aqueous ozone revealed the highest level of biocompatibility compared with commonly used antiseptics. Ozone should be used to help combat the microorganisms associated with infected root canals (44). Also, ozone dissolved in water is more biocompatible and less cytotoxic to oral cells than sodium hypochlorite. The application of ozone gas to surface caries lesions (with or without sealing) or infected root canals demonstrably improves clinical findings. The potential effect of ozone in the treatment of periodontitis, periimplantitis and endodontal infections is well known but it needs to be investigated in further controlled studies (29).

In our study, the HealOzone treatment showed superior effect to mehanical and NaOCl irrigation in eliminating intracanal aerobic and anaerobic bacteria. Although the time period of 40 seconds of ozone application caused reduction in aerobic, anaerobic and total number of bacteria, it is quesklinička ispitivanja kako bi se dokazao točan način njegova djelovanja i primjene (29).

U ovom istraživanju je primjena HealOzona pokazala snažan antimikrobni učinak i redukciju u broju aerobnih i anaerobnih bakterija u odnosu na bakterije izolirane nakon mehaničke obrade i ispiranja kanala NaOCl-om. Iako je vrijeme primjene ozona od 40 sekundi bilo dovoljno za smanjenje aerobnih, anaerobnih i ukupnog broja bakterija, pitanje je bi li dulja primjena bila dovoljna za potpunu redukciju bakterija u svim uzorcima. Zbog dodatnog smanjenja bakterija u kanalima nakon primjene ozona, ova se metoda preporučuje za kliničku primjenu. Potrebna su daljnja istraživanja kako bi se dokazao točan učinak plina ozona tijekom primjene u korijenskim kanalima.

Zaključak

Mehanička i kemijska obrada korijenskih kanala, te dodatna primjena ozona pokazali su se uspješnima u smanjivanju broja aerobnih i anerobnih bakterija u korijenskom kanalu. Korištenjem ozona, kao snažnog antimikrobnog sredstva, znatno je smanjen broj bakterija te se njegova primjena preporučuje kao dodatna metoda dezinfekcije pri obradi korijenskih kanala.

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tionable whether longer exposure time could provide complete eradication in all samples. Because of the improvements in bacterial reduction after additional application of ozone in the root canals, this procedure is recommended for use in clinical practice. Further studies are necessary to characterize the precise effect of ozone gas for endodontic root canal treatment.

Conclusion

The chemomechanical and additional intracanal ozone application succeeded in reducing root canal infection and had the capacity to eradicate both aerobic and anaerobic bacteria presented in infected root canals. Application of ozone as a powerful antimicrobial agent has led to a significant reduction of bacteria so it can be used as additional method in root canal treatment.

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Conflict of interest

The authors do not have any financial interest in the companies whose materials are included in this article.

Abstract

Aim: The aim of this study was to determine the amount of aerobic, anaerobic, and total number of bacterial colonies after chemomechanical root canal treatment and after additional intracanal disinfection using ozone. **Materials and Methods:** Twenty - three patients with the same number of single rooted teeth (n=23) with periapical lesion (<5 mm) observed on retroalveolar radiogram or panoramic radiograph took part in this study. For the microbiological analysis three swabs were taken: immediately before (B1), after mechanical instrumentation and irrigation with 2.5% sodium hypochlorite (B2) and after chemomechanical treatment and additional intracanal ozone application (B3). Swabs were taken with sterile paper points, bacteria were cultivated and the results were interpreted after 14 days using the API 20A system. The data were analyzed by Wilcoxon Signed Rank Test measuring the number of bacterial colonies before and after the root canal treatment. **Results:** The difference between groups B1 and B2, B1 and B3 and B2 and B3 for all three groups: aerobic bacteria, anaerobic bacteria and total number of bacteria was statistically significant ($p<0.05$). **Conclusion:** Both methods showed a strong effect in bacterial number reduction. After the chemomechanical and additional ozone root canal treatment, the number of aerobic and anaerobic bacterial colonies further decreased compared to the measurements immediately before and after the chemomechanical root canal treatment. Because of the improvements in bacterial reduction after additional application of ozone in the root canal, this procedure is recommended for use in clinical practice.

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Anti-bacterial Agents; Root Canal Preparation; Ozone; Disinfection

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