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Evaluation of the Antibacterial Effects of Single and Combined use of Different Irrigation Solutions Against Intracanal *Enterococcus Faecalis*

Prosudba antibakterijskog učinka pojedinačne i kombinirane uporabe otopina za ispiranje korijenskih kanala na *Enterococcus faecalis*

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Abstract

Objectives: This study assessed the antibacterial activity of both separate and combined uses of 5.25% sodium hypochlorite (NaOCl), 2% chlorhexidine (CHX), 17% ethylenediaminetetraacetic acid (EDTA), 3% hydrogen peroxide (H₂O₂), MTAD, SmearClear (SC) and 13.8% chlorine dioxide (ClO₂) irrigation solutions against *Enterococcus faecalis*. **Material and Methods:** Two hundred eighty single rooted human premolars were randomly grouped into 26 test and 2 control (negative and positive) groups and were incubated for 24 h with *E. faecalis*, except for the negative control group. The tested solutions were as follow: NaOCl; CHX; ClO₂; MTAD; SC; EDTA; H₂O₂; NaOCl + CHX; NaOCl + MTAD; SC + NaOCl; EDTA + NaOCl; H₂O₂ + NaOCl; ClO₂ + CHX; CHX + MTAD; SC + CHX; EDTA + CHX; CHX + H₂O₂; ClO₂ + MTAD; SC + ClO₂; EDTA + ClO₂; ClO₂ + H₂O₂; SC+MTAD; EDTA+MTAD; MTAD + H₂O₂; SC + H₂O₂; and EDTA + H₂O₂. Optic density values were recorded at 0, 6, 12, 18, 24, 30, 36, 42 and 48 h and bacterial growth curve created for each solution. **Results:** The CHX, MTAD and ClO₂ showed a high potential for the elimination of *E. faecalis*, both alone and in all combinations. The EDTA, H₂O₂, H₂O₂ + EDTA, H₂O₂ + NaOCl and SC + NaOCl groups showed less antibacterial activity than the other groups. The SC + CHX group showed the best antibacterial effect against *E. faecalis*. **Conclusion:** The SC + CHX combination can be recommended as the most effective irrigation regimen against *E. faecalis* in persistent endodontic infections.

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Key words

Root Canal Irrigants; Local Anti-Infective Agents; *E. faecalis*

Introduction

Microorganisms are one of the significant etiological factors in the pathogenesis of periapical tissue diseases. For this reason, it is very important to completely remove microorganisms from the infected root canals during a root canal treatment. Some *ex vivo* and clinical studies have reported that there are some untouched areas in the root canal walls during mechanical preparation. Therefore, to combine mechanical instrumentation with chemical irrigation is extremely important (1,2,3) in elimination of the microorganisms known to be extremely resistant against antimicrobial agents, such as *Enterococcus (E.) faecalis* (4).

The relationship of *E. faecalis* with different forms of periradicular infections, including primary and permanent infec-

Uvod

Mikroorganizmi su jedan od važnijih etioloških čimbenika u patogenezi bolesti periapikalnog tkiva. To je razlog da je tijekom endodontskog liječenja vrlo važno potpuno ukloniti mikroorganizme iz zaraženih korijenskih kanala. U nekim istraživanjima *ex vivo* i kliničkim studijama autori su pokazali da postoje mnoga nedirnuta područja na stijenkama korijenskog kanala tijekom mehaničke pripreme. Zato je kombiniranje mehaničkih instrumenata s kemijskom irigacijom itekako važno (1, 2, 3) u uklanjanju mikroorganizama za koje se zna da su iznimno otporni na antimikrobna sredstva, kao što je *Enterococcus (E.) faecalis* (4).

Odnos bakterije *E. faecalis* s različitim oblicima periradikalnih infekcija, uključujući primarne i kronične infekcije,

tions, is well known because of its ability to grow in the presence or absence of oxygen. *E. faecalis* has been reported to be associated more with asymptomatic chronic periradicular lesions rather than acute periradicular periodontitis or acute periradicular abscesses in the category of primary endodontic infections (5, 6). It has been confirmed that *E. faecalis* is significantly associated with treatment failures. Whereas this species was detected in 18% of the cases of primary endodontic infections, its prevalence in root-filled teeth was much higher: 67% of the cases (7).

Different endodontic irrigation solutions and disinfection techniques have been introduced to decrease the root canal bacterial count. The main effect of irrigation is to both physically and chemically remove organic and inorganic debris, infected materials and soft and hard tissue residues from the root canals. In this way, these materials are inhibited from accumulating, clogging and becoming inaccessible in the apical section of the root canals. Endodontic irrigation solutions usually have antibacterial properties. It has been proven that a combined use of irrigation materials with a different antimicrobial spectrum can even increase the antibacterial activity provided by a single solution through a synergic and/or additive effect for the elimination of hundreds of types of microorganisms forming on the root canal microflora (5).

There is a limited number of studies in the literature assessing and comparing the combined use of frequently used endodontic irrigation solutions in terms of their antibacterial characteristics. The positive or negative effects of the antibacterial interactions of current and potential irrigation solutions will especially guide the treatment of persistent endodontic infections. Therefore, understanding the interactions between irrigation solutions will be very helpful in implementing the most effective treatment procedure for persisting infections in endodontic cases. This current study aimed to investigate the antibacterial activity of both the single and combined uses of sodium hypochlorite (NaOCl), chlorhexidine (CHX), ethylenediaminetetraacetic acid (EDTA), hydrogen peroxide (H₂O₂), BioPure MTAD (Densply, Tulsa Dental, Tulsa, OK, USA), SmearClear (SC) (Sybron Endo, Orange, CA, USA) and chlorine dioxide (ClO₂) against *E. faecalis* and to compare the antibacterial effects of this study's solutions against *E. faecalis*.

Material and Methods

The current study was started after receiving approval from the Ethical Board of Selcuk University, Faculty of Dentistry (Document # 203). One hundred eighty single-rooted human lower premolars that had been extracted for orthodontic or periodontal reasons were used. The hard and soft tissue residuals on the teeth were cleaned with curettes and then the teeth were kept at 4° C in a 100 % moist environment until the laboratory procedures were performed.

The teeth were de-coronated with a diamond disk (Ortho Technology Inc., Tampa, FL, USA) under water cooling to a standardized root length of 14 ± 0.5 mm. The canal lengths were standardized with 15 K-File hand devices (Mani Inc., Tochigi, Japan) and enlarged with ProTaper Universal NiTi

dobro je poznat zbog njegova svojstva rasta kako u prisutnosti kisika, tako i u njegovoj odsutnosti. Za *E. faecalis* dokazano je da je više povezan s asimptomatskim kroničnim periradikularnim lezijama, a ne s akutnim periradikularnim parodontitisom ili akutnim periradikularnim apscesima u kategoriji primarnih endodontskih infekcija (5, 6). Potvrđeno je da je ta bakterija značajno povezana s neuspjehom liječenja. Iako je ta vrsta otkrivena u 18 % slučajeva primarnih endodontskih infekcija, njezina prevalencija u endodontski liječenim zubima bila je mnogo veća – 67 % slučajeva (7).

Predstavljene su različite otopine za endodontsku irigaciju i tehnike dezinfekcije kako bi se smanjio broj bakterija u korijenskim kanalima. Glavni učinak irigacije jest fizičko i kemijsko uklanjanje organskih i anorganskih nečistoća, zaraženih materijala i ostataka mekoga i tvrdoga tkiva iz korijenskih kanala. Na taj se način sprječava nakupljanje i začepljenje tim materijalima koji onemogućuju pristup apikalnom dijelu korijenskih kanala. Otopine za endodontsku irigaciju obično imaju antibakterijska svojstva. Dokazano je da kombinirana upotreba otopina za irigaciju različitoga antimikrobnog spektra može čak povećati antibakterijsko djelovanje koje ima pojedinačna otopina sinergijskim i/ili dodatnim učinkom za uklanjanje stotina vrsta mikroorganizama koji se formiraju u mikroflori korijenskog kanala (5).

Pregledom dosadašnje literature ustanovljen je ograničen broj studija u kojima se procjenjuje i uspoređuje kombinirana upotreba često korištenih endodontskih otopina za irigaciju, kad je riječ o njihovim antibakterijskim svojstvima. Pozitivni ili negativni učinci antibakterijskih interakcija postojećih i potencijalnih otopina za irigaciju posebno će usmjeravati liječenje perzistentnih endodontskih infekcija. Zato će razumijevanje interakcije između otopina za irigaciju biti od velike pomoći u provedbi najučinkovitijeg postupka liječenja perzistentnih infekcija u endodontskim slučajevima. Ova studija imala je za cilj istražiti antibakterijsko djelovanje pojedinačnih i kombiniranih primjena natrijeva hipoklorita (NaOCl), klorheksidina (CHX), etilendiamintetraoctene kiseline (EDTA), vodikova peroksida (H₂O₂), BioPure, MTAD-a (Densply, Tulsa Dental, Tulsa, OK, SAD), SmearCleara (SC) (Sybron Endo, Orange, CA, SAD) i klorova dioksida (ClO₂) na *E. faecalis* i usporediti antibakterijske učinke svih korištenih otopina u studiji.

Materijali i metode

Ovo istraživanje započeto je nakon što ga je odobrilo Etičko povjerenstvo Stomatološkog fakulteta Sveučilišta Selcuk (dokument br. 203). Korišteno je stotinu i osamdeset jednokorijenskih ljudskih donjih pretkutnjaka (premolara) izvađenih iz ortodontskih ili parodontskih razloga. Ostaci tvrdoga i mekoga tkiva na zubima očišćeni su kiretama, a zatim su do laboratorijskih postupaka zubi čuvani na temperaturi od 4 °C u 100 % vlažnom okruženju.

Zubi su dekoronirani dijamantnim diskom (Ortho Technology Inc., Tampa, FL, SAD) uz vodeno hlađenje do standardizirane dužine korijena od 14 ± 0,5 mm. Dužine kanala standardizirane su ručnim instrumentom K-File #15 (Mani Inc., Tochigi, Japan) i proširene su ProTaper univerzal-

rotary files (Dentsply, Tulsa Endodontics, Tulsa, OK, USA) using the crown-down method. The apical parts of the root canals were finished at F3. During the preparations, the root canals were irrigated with 1 ml of 5.25 % NaOCl solution (Caglayan Kimya San., Konya, Turkey).

In order to remove the smear layer of root canal walls, the roots were exposed to an ultrasonic bath (USG 4000 Ultraschall, Dentaaurum, Ispringen, Germany) in 17 % EDTA (AppliChem GmbH, Darmstadt, Germany), 5.25 % NaOCl and distilled water for 10 min in each solution, in that order (8). The samples were then embedded perpendicular to the long axes in a silicone impression material (Zetaplus, Zhermack SpA, Badia Polesine (RO), Italy) and placed in metal plates filled with distilled water and capped, 10 in each group, and sterilized at 121° C for 20 min in an autoclave (Hirayama, Saitama, Japan). The metal plates were then opened inside a Biosafety Level 2 (BSL 2) lamina air-flow cabin and each sample was coated with two layers of nail polish (Loreal Jet-Set Diamond, Paris, France) in order to prevent bacterial leak during the experimental procedures. In order to contaminate the sterilized root canals with *E. faecalis* for experimental purposes, fresh cultures of *E. faecalis* (ATCC 29212) microorganisms were obtained after 24 h of incubation inside Brain Heart Infusion Broth (BHI) (bioMerieux® sa 69280, Marcy l'Etoile, France). The optical density (OD) of the *E. faecalis* suspension inside BHI was adjusted according to McFarland No: 0.5 standard to approximately 1.5×10^8 colony/ml. Except for the negative control group, the *E. faecalis* suspension was planted to the root canals with the help of a sterile 1 ml tuberculin syringe. Then, the samples in the metal plates were incubated at 37° C for 24 h. After incubation, the metal plates were opened in a BSL 2 air-flow cabin and irrigation was performed with the experimental solutions.

A flow chart of the study design is shown in Figure 1. The basic irrigation solutions and their combinations used in the study are presented in Table 1. All solutions except for EDTA were ready to use. The 17 % EDTA solution was prepared in the laboratory according to the instructions of Sen et al. (9).

Each root canal was irrigated with a single or combined irrigation solution for 5 min using 30-gauge endodontic irrigation needles (KerrHawe SA, Bioggio, Switzerland), according to the irrigation regimens indicated in Figure 1. Sterile F3 paper cones were placed into the root canals for 1 min to allow for complete absorption. The paper cones were then put into sterile tubes containing 1 ml BHI Broth and placed into a vortex device (MS 1 Minishaker IKA®, Darmstadt, Ger-

nim nikal-titanijskim rotacijskim instrumentima (Dentsply, Tulsa Endodontics, Tulsa, OK, SAD) primjenom tzv. metode *crown-down*. Obrada apikalnih dijelova korijenskih kanala završena je instrumentom F3. Tijekom preparacije, korijenski kanali irigrani su 1 mL 5,25-postotne otopine natrijeva hipoklorita (Caglayan Kimya San., Konja, Turska).

Kako bi se uklonio zaostadni sloj sa stijenki korijenskih kanala, korijeni su bili izloženi ultrazvučnoj kupki (USG 4000 Ultraschall, Dentaaurum, Ispringen, Njemačka) u 17-postotnoj EDTA-i (AppliChem GmbH, Darmstadt, Njemačka), 5,25-postotnom natrijevu hipokloritu i destiliranoj vodi po 10 minuta u svakoj otopini, tim redosljedom (8). Uzorci su zatim ugrađeni okomito na uzdužnu os u silikonski otisni materijal (Zetaplus, Zhermack SpA, Badia Polesine (RO), Italija) te stavljani u metalne ploče napunjene destiliranom vodom i zatvorene, po 10 u svakoj skupini, i 20 minuta sterilizirane na 121 °C u autoklavu (Hirayama, Saitama, Japan). Zatim su metalne ploče otvorene u kabini za laminaciju zraka na razini biološke sigurnosti (BSL 2) i svaki je uzorak premazan dvama slojevima laka za nokte (Loreal Jet-Set Diamond, Pariz, Francuska) da bi se spriječilo istjecanje bakterija tijekom pokusnog procesa. Kako bi se za pokusne svrhe kontaminirali sterilizirani korijenski kanali bakterijom *E. faecalis*, svježe kulture mikroorganizama *E. faecalis* (ATCC 29212) dobivene su nakon 24-satne inkubacije u juhi od moždane supstancije tj. medij Brain Heart Infusion Broth – BHI (bioMerieux® sa 69280, Marcy l'Etoile, Francuska). Optička gustoća (OG) suspenzije *E. faecalis* unutar BHI-ja podešena je u skladu s McFarlandovim No: 0,5 standardom do približno $1,5 \times 10^8$ kolonijel/mL. Uz negativnu kontrolnu skupinu, suspenzija *E. faecalis* postavljena je u korijenske kanale s pomoću sterilne 1 mL tuberkulinske štrcaljke. Zatim su uzorci u metalnim pločama inkubirani 24 sata na temperaturi od 37 °C. Nakon inkubacije metalne ploče su otvorene u kabini s protokom zraka BSL 2 i irigacija je obavljena s pokusnim otopinama.

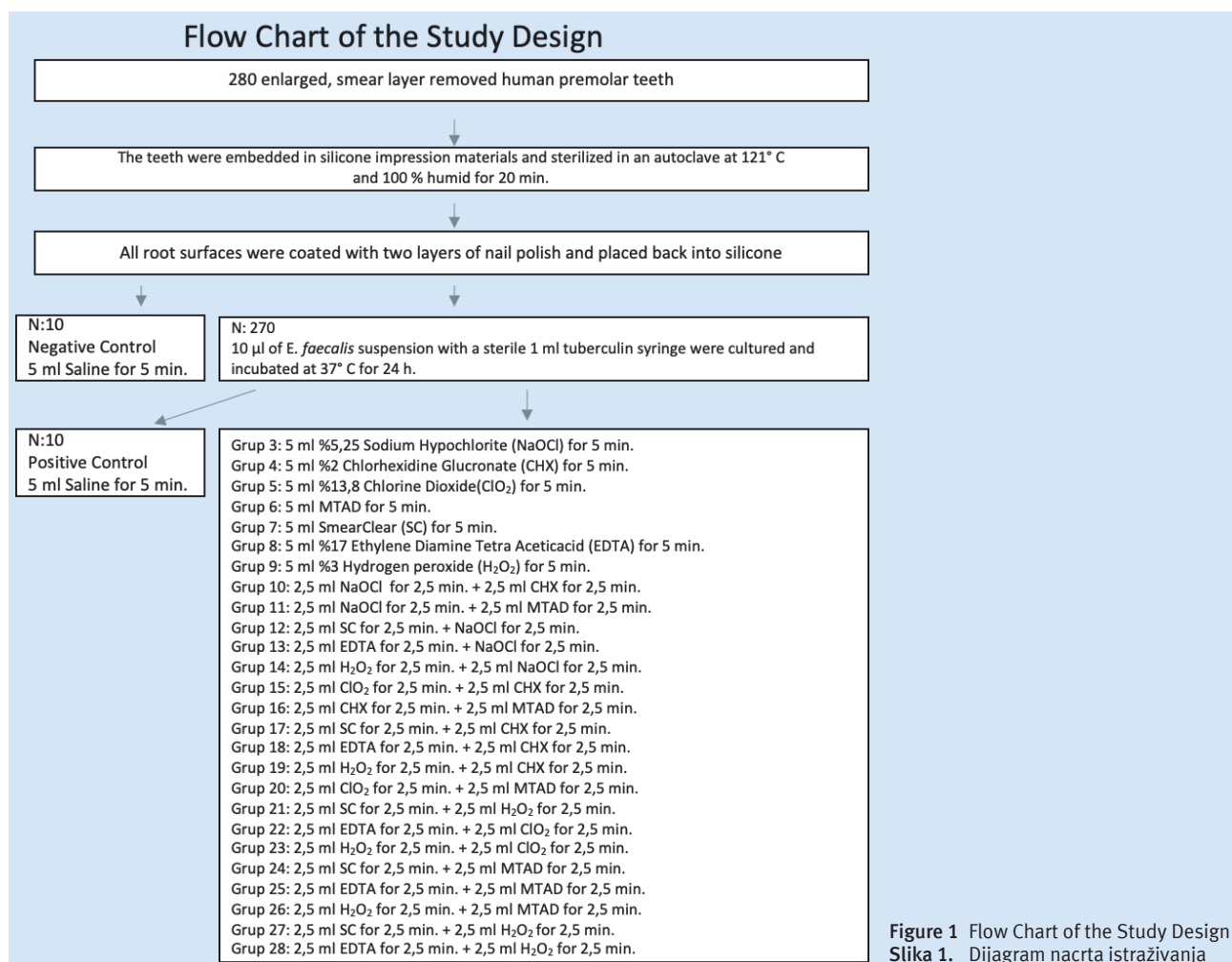
Shema dijagrama dizajna studije prikazana je na slici 1. Osnovne otopine za irigaciju i njihove kombinacije korištene u studiji nalaze se u tablici 1. Sve otopine, osim EDTA-e, bile su spremne za upotrebu. 17-postotna otopina EDTA-e pripremljena je u laboratoriju prema uputama Sena i suradnika. (9).

Svaki je korijenski kanal irigran jednom ili kombiniranom otopinom u trajanju od 5 minuta s pomoću endodontskih igala promjera 30 za vlaženje korijenskih kanala (KerrHawe SA, Bioggio, Švicarska) prema protokolima irigacije navedenima na slici 1. Zatim su u korijenske kanale jednu minutu stavljeni sterilni papirnati štapići F3 da bi se omogu-

Table 1 Basic irrigation solutions and manufacturers

Tablica 1. Osnovne irigacijske otopine i proizvođači

The Basic Irrigation Solutions • Osnovne irigacijske otopine	Manufacturer • Proizvođač
5.25 % Sodium hypochlorite (NaOCl) • 5,25-postotni natrijev hipoklorit (NaOCl)	Caglayan Kimya San., Konya/Turkey • Turska
2 % Chlorhexidine gluconate (CHX) • 2-postotni klorheksidin-glukonat (CHX)	Klorhex, Drogosan ilač san., Ankara/Turkey • Turska
13.8 % Chlorine dioxide (ClO ₂) • 13,8-postotni klorni dioksid (ClO ₂)	Bioclenz, Frontier Pharmaceutical, Melville, NY, USA • SAD
BioPure MTAD (MTAD)	Dentsply, Tulsa Dental, Tulsa, OK, USA • SAD
SmearClear (SC)	Sybron Endo, Orange, CA, USA • SAD
3 % Hydrogen peroxide (H ₂ O ₂) • 3-postotni vodikov peroksid (H ₂ O ₂)	Kimpa ilač lab. Ve tic. Ltd. sti.; Istanbul, Turkey • Turska
17 % Ethylene Diamine Tetra Acetic Acid (EDTA) • 17-postotna etilendiamintetraoctena kiselina (EDTA)	Prepared in the laboratory • Pripremljeno u laboratoriju



many) for 5 min. A 200 µl sample was taken from the shaken medium and transferred to a well in a 96-well sterile ELISA plate (Costar 3599, Corning, NY, USA). Each sample went through this procedure twice for a total of two wells per sample to get averages for the measurements. The plates were then placed in an ELISA reader (BioTek ELx800, Absorbance Microplate Reader, Winooski, VT, USA) to complete the first optic density (OD) test (hour 0) at a wavelength of 450 nm, and the data were recorded. The data were taken every 6 h in the ELISA reader and repeated twice. The plates were placed in the incubator and kept at 37° C in a 100 % moist environment during the experiment. Data were obtained for each sample at hours 0, 6, 12, 18, 24, 30, 36, 42 and 48. The averages of the data collected at each measurement were calculated separately for each group, and an average OD value was determined for each period. Average OD data obtained for each sample at each time period were used, and a time-dependent OD change graph was created for each experimental group (Figure 2,4–10)

For statistical analysis, the Kruskal–Wallis and Mann–Whitney U tests were used to find any significant differences among the study groups. Significance level was accepted as $p > 0.05$.

čila potpuna apsorpcija. Nakon toga su papirnati štapići odloženi u sterilne epruvete s 1 mL medija BHI i stavljani 5 minuta u vibrirajući uređaj (MS 1 Minishaker IKA®, Darmstadt, Njemačka). Uzorak od 200 µL uzet je iz protresenog medija i premješten u jažicu u sterilnoj ELISA ploči s 96 jažica (Costar 3599, Corning, NY, SAD). Svaki je uzorak dvaput prošao taj postupak za ukupno dvije jažice po uzorku da bi se dobili prosjeci za mjerenja. Ploče su zatim stavljene u čitač ELISA-e (BioTek EL x 800, čitač apsorpcijskih mikroploča, Winooski, VT, SAD) kako bi se završio prvi test optičke gustoće (OG) (sat 0) pri valnoj dužini od 450 nm. Dobiveni podatci su zabilježeni. Iz čitača ELISA-e podatci su uzimani svakih 6 sati i ponovljeni dva puta. Tijekom pokusa ploče su stavljene u inkubator i držane na temperaturi od 37 °C u 100 % vlažnom okruženju. Podatci su dobiveni za svaki uzorak u razdoblju od 0, 6, 12, 18, 24, 30, 36, 42 i 48 sati. Prosječni podatci dobiveni pri svakom mjerenju izračunati su zasebno za svaku skupinu, a prosječna vrijednost OG-a utvrđena je za svako razdoblje. Korišteni su prosječni OG podatci dobiveni za svaki uzorak za svako razdoblje, a kreiran je vremenski ovisan grafikon promjene OG-a za svaku pokusnu skupinu (slika 2., 4. – 10.)

Za statističku analizu korišteni su Kruskal–Wallisov test i Mann–Whitneyjev U-test kako bi se utvrdile razlike među ispitivanim skupinama. Razina značajnosti prihvaćena je na $p > 0,05$.

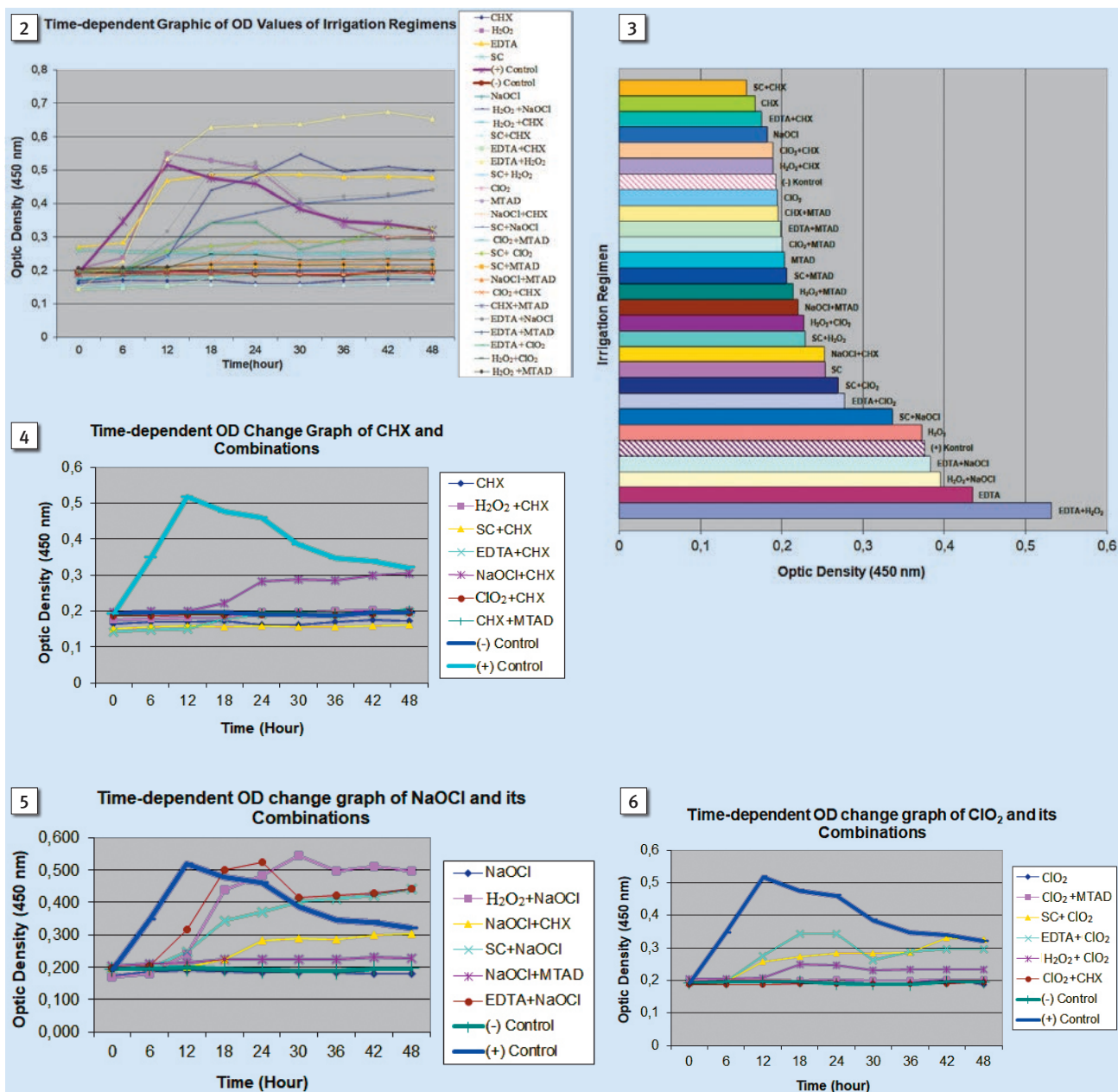


Figure 2 Bacterial growth curves resulting from time-dependent OD values
 Slika 2. Krivulje rasta bakterija koje su rezultat vremenski ovisnih OG vrijednosti
 Figure 3 Mean OD values of irrigation regimens used in the experiment
 Slika 3. Srednje vrijednosti OG-a za irigaciju korištene u pokusu
 Figure 4 CHX and Combinations
 Slika 4. CHX i kombinacije
 Figure 5 NaOCl and Combinations
 Slika 5. NaOCl i kombinacije
 Figure 6 ClO₂ and Combinations
 Slika 6. ClO₂ i kombinacije

Results

The post-incubation, time-dependent OD values (at 450 nm) of the samples tested for antibacterial activity following single and combined uses of the root canal solutions are shown in Figure 2.

Overall statistical results showed that significant similarity was found between the OD values of samples taken from root canals irrigated with CHX, NaOCl, MTAD, SC and

Rezultati

Postinkubacija i OG vrijednosti uzoraka ovisnih o vremenu (na 450 nm) testiranih na antibakterijsko djelovanje nakon pojedinačnih i kombiniranih primjena otopina korijenskog kanala prikazani su na slici 2.

Ukupni statistički rezultati pokazali su da je pronađena značajna sličnost između OG vrijednosti uzoraka uzetih iz korijenskih kanala irigiranih CHX-om, NaOCl-om, MTAD-

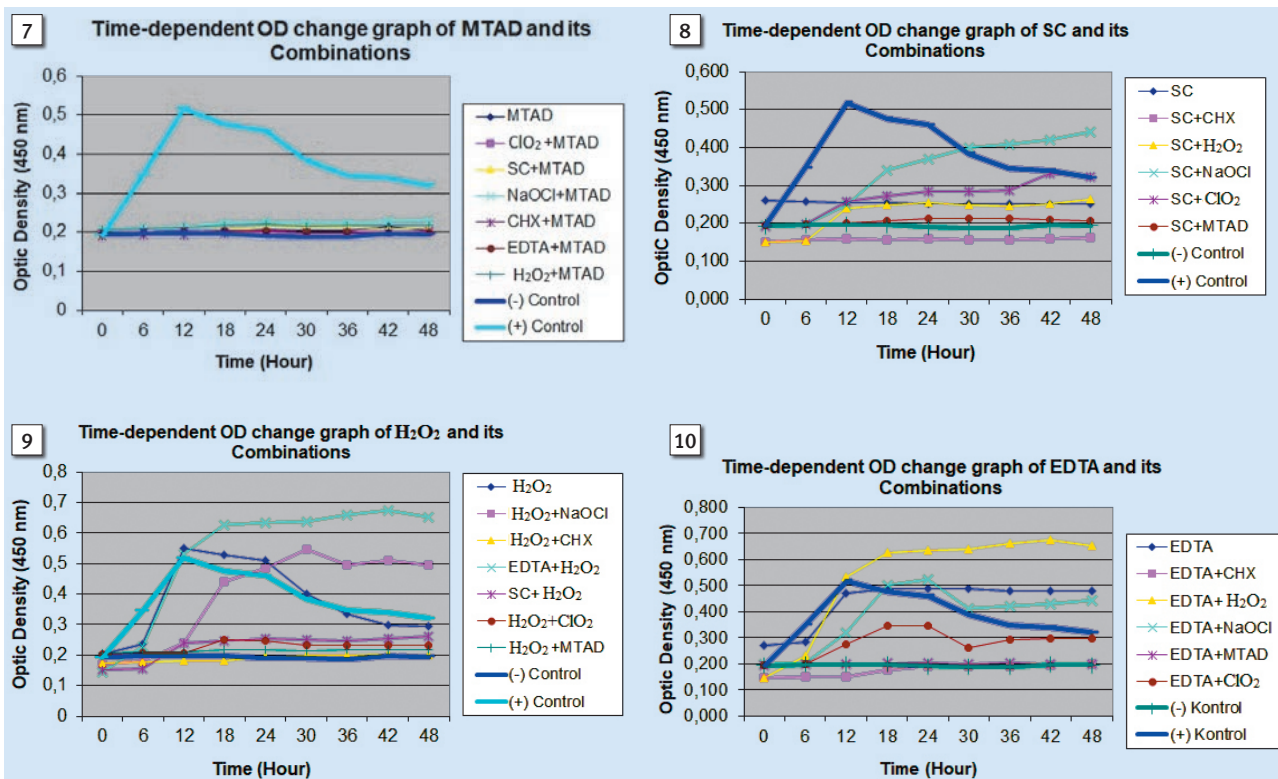


Figure 7 MTAD and Combinations

Slika 7. MTAD i kombinacije

Figure 8 SC and Combinations

Slika 8. SC i kombinacije

Figure 9 H₂O₂ and CombinationsSlika 9. H₂O₂ i kombinacije

Figure 10 EDTA and Combinations

Slika 10. EDTA i kombinacije

ClO₂ and the negative control group ($p > 0.05$). On the other hand, the positive control group showed significant similarities with OD values of the samples taken from the root canals irrigated with H₂O₂ and EDTA ($p > 0.05$).

CHX and CHX combinations

No statistically significant difference was found between the negative control group and CHX and its combinations at all times, including the NaOCl + CHX group, which showed an increase in OD values (Figure 2, 4) ($p > 0.05$).

NaOCl and NaOCl combinations

No statistically significant difference was found between the NaOCl and NaOCl + MTAD groups and the negative control group at all times ($p > 0.05$). The values of samples irrigated with H₂O₂ + NaOCl and EDTA + NaOCl at hours 24, 36 and 48 were found to be similar with the positive control group ($p > 0.05$) (Figure 5). The increase in the OD value of the SC + NaOCl group after hour 6 was found to be significantly different from the negative control group after hour 36 ($p < 0.05$) (Figure 5).

ClO₂ and ClO₂ combinations

ClO₂ and all its combinations were found to be significantly similar with the negative control group at all times ($p > 0.05$) (Figure 6).

om, SC-om i ClO₂ i negativne kontrolne skupine ($p > 0,05$). S druge strane, pozitivna kontrolna skupina imala je značajne sličnosti s vrijednostima OG uzoraka uzetih iz korijenskih kanala irigriranih otopinama H₂O₂ i EDTA ($p > 0,05$).

CHX i njegove kombinacije

Nije pronađena statistički značajna razlika između negativne kontrolne skupine i CHX-a i njegovih kombinacija u svim razdobljima, uključujući skupinu NaOCl + CHX koja je pokazala porast vrijednosti OG-a (slike 2., 4.) ($p > 0,05$).

NaOCl i njegove kombinacije

Nije nađena statistički značajna razlika između skupina NaOCl i NaOCl + MTAD te negativne kontrolne skupine u svakom trenutku ($p > 0,05$). Vrijednosti uzoraka irigriranih otopinama H₂O₂ + NaOCl i EDTA + NaOCl tijekom 24, 36 i 48 sati slične su pozitivnoj kontrolnoj skupini ($p > 0,05$) (slika 5.). Pokazalo se da se porast OG vrijednosti u skupini SC + NaOCl nakon jednog sata značajno razlikuje od negativne kontrolne skupine nakon 36 sati ($p < 0,05$) (slika 5.).

ClO₂ i njegove kombinacije

ClO₂ i sve njegove kombinacije vrlo su slične negativnoj kontrolnoj skupini u svakom trenutku ($p > 0,05$) (slika 6.).

MTAD and MTAD combinations

No difference was found between OD values of MTAD and all its combinations and the negative control group ($p > 0.05$). (Figure 7).

SC and SC combinations

SC, SC + CHX, SC + MTAD and SC + H₂O₂ were found to be statistically similar with the negative control group at all times ($p > 0.05$) (Figure 8). A continuous increase was found in the OD values of the groups irrigated with SC + ClO₂ and SC + NaOCl at all time periods (Figure 8). While this increase was not found to be statistically significant in the group irrigated with SC + ClO₂ ($p > 0.05$), in the group irrigated with SC + NaOCl, it was found to be significantly different for the measurements at hours 30, 36 and 48 when compared with the negative control group ($p < 0.05$).

H₂O₂ and H₂O₂ combinations

There were no statistically significant differences between the OD values of the groups irrigated with SC + H₂O₂, H₂O₂ + ClO₂, H₂O₂ + CHX and H₂O₂ + MTAD and those of the negative control group (Figure 9). The group irrigated with H₂O₂ was found to be statistically significantly similar with the positive control group ($p > 0.05$). The increase in the first 24-hour period in the groups irrigated with H₂O₂ + EDTA and H₂O₂ + NaOCl was not statistically significant when compared with the positive control group ($p > 0.05$) (Figure 9). However, the increase continuing at hours 24 and 48 in the groups irrigated with H₂O₂ + EDTA and H₂O₂ + NaOCl was significantly different when compared with the positive control group ($p < 0.05$) (Figure 9).

EDTA and EDTA combinations

The time-dependent increase in OD values of the groups irrigated with EDTA and EDTA + H₂O₂ was found to be significantly similar with the positive control group ($p > 0.05$) (Figure 10). The increase observed for the first 6 hours in the EDTA + H₂O₂ group was found to be significantly similar with the negative control group ($p > 0.05$), while that increase was found to be significantly similar with the positive control group at hours 6 and 24 ($p > 0.05$). In the remaining periods, the increase in the OD values was found to be significantly different from those of the positive control group ($p < 0.05$). The OD values of the group irrigated with EDTA + ClO₂ were found to be similar with the negative control group at all times ($p > 0.05$) (Figure 10). When the time-dependent OD value of EDTA + NaOCl was compared with the negative control group, it was found to be significantly different after hour 18 ($p < 0.05$) (Figure 10). However, no statistically significant difference was observed between the groups irrigated with EDTA + CHX and EDTA + MTAD and the negative control group ($p > 0.05$) (Figure 10).

Discussion

In this study, the bacterial growth in samples from infected root canals irrigated with antibacterial irrigation solutions was compared with normal bacterial growth (positive control group), and bacterial growth from when the sterilized roots

MTAD i njegove kombinacije

Nije dokazana razlika između OG vrijednosti MTAD-a i svih njegovih kombinacija i negativne kontrolne skupine ($p > 0,05$) (slika 7.).

SC i njegove kombinacije

SC, SC + CHX, SC + MTAD i SC + H₂O₂ statistički su slični negativnoj kontrolnoj skupini u svakom trenutku ($p > 0,05$) (slika 8.). Utvrđeno je kontinuirano povećanje vrijednosti OG-a u skupinama irigranim otopinama SC + ClO₂ i SC + NaOCl u svim razdobljima (slika 8.). Iako se nije ustanovilo da je taj porast statistički znan u skupini koja je irigrana otopinom SC + ClO₂ ($p > 0,05$), u skupini irigranoj otopinom SC + NaOCl ustanovljeno je da se značajno razlikuje za mjerenja u satima 30, 36 i 48 u usporedbi s negativnom kontrolnom skupinom ($p < 0,05$).

H₂O₂ i njegove kombinacije

Nije bilo statistički značajnih razlika između OG vrijednosti skupina irigranih otopinama SC + H₂O₂, H₂O₂ + ClO₂, H₂O₂ + CHX i H₂O₂ + MTAD i vrijednosti negativne kontrolne skupine (slika 9.). Uočeno je da je skupina irigrana otopinom H₂O₂ statistički značajno slična pozitivnoj kontrolnoj skupini ($p > 0,05$). Povećanje u prva 24 sata u skupinama irigranim otopinama H₂O₂ + EDTA i H₂O₂ + NaOCl nije bilo statistički značajno u usporedbi s pozitivnom kontrolnom skupinom ($p > 0,05$) (slika 9.). No porast koji se nastavio tijekom 24 i 48 sati u skupinama irigranim otopinama H₂O₂ + EDTA i H₂O₂ + NaOCl bio je značajno drukčiji u usporedbi s pozitivnom kontrolnom skupinom ($p < 0,05$) (slika 9.).

EDTA i njezine kombinacije

Otkriveno je da vremenski ovisan porast vrijednosti OG grupe irigrane otopinama EDTA i EDTA + H₂O₂ značajno slični pozitivnoj kontrolnoj skupini ($p > 0,05$) (slika 10.). Pokazalo se da je porast zabilježen u prvih 6 sati u skupini EDTA + H₂O₂ značajno sličan negativnoj kontrolnoj skupini ($p > 0,05$), a ustanovljeno je da je taj porast vrlo sličan pozitivnoj kontrolnoj skupini nakon 6 i 24 sata ($p > 0,05$). U preostalim razdobljima uočeno je da se porast vrijednosti OG-a značajno razlikuje od vrijednosti pozitivne kontrolne skupine ($p < 0,05$). Istaknuto je da su OG vrijednosti za skupinu irigranu otopinom EDTA + ClO₂ u svakom trenutku slične negativnoj kontrolnoj skupini ($p > 0,05$) (slika 10.). Kad se usporedila vremenski ovisna OG vrijednost EDTA + NaOCl-a s negativnom kontrolnom skupinom, ustanovljeno je da se značajno razlikuju nakon 18 sati ($p < 0,05$) (slika 10.). No nije opažena statistički značajna razlika između skupina irigranih otopinama EDTA + CHX i EDTA + MTAD i negativne kontrolne skupine ($p > 0,05$) (slika 10.).

Rasprava

U ovom istraživanju je rast bakterija u uzorcima iz zaraženih kanala korijena koji se vlaže antibakterijskim otopinama za irigaciju uspoređen s normalnim rastom bakterija (pozitivna kontrolna skupina) i rastom bakterija nakon inkubaci-

were incubated (negative control group). During the incubation period, it was clearly determined in which period the reproduction occurred, slowed down and regressed.

The antibacterial activity of irrigation solutions is known to increase with the increase in volume and application time of the irrigation (10, 11). In this study, the standard irrigation application time was determined to be a total of 5 min of irrigation with a 5 ml solution for all canals. In combined uses, the total volume used was 2.5 ml + 2.5 ml for each solution. For MTAD, the manufacturer recommended an application regimen of 5 ml for each canal. Thus, MTAD also used the same volume and time interval as the other solutions, making all solutions in the experiment comparable. All of the root canals were also irrigated at a post-experiment stage with 5ml saline to achieve maximum dilution of the residual solution. In this way, the impact of the transferred solution on the medium was minimized and the residual antibacterial effect was reduced.

In this study, the order of the solution application recommended by researchers was used (12, 13). It is acknowledged that a residual antimicrobial effect might still be present unless the activity of the solution is neutralized by means of an inactivator (14). However, to be able to standardize the experiments undertaken in this study, neutralization of the test solutions was not carried out. One of the other reasons for this is that some of the test solutions, such as MTAD and SC, are proprietary products and there are no chemical inactivators known to exist for them. Therefore, to achieve uniformity in the experiment methodology and to make it an easier procedure to compare the relatively high number of solutions, none of the test specimens were inactivated.

It has been reported by many researchers that CHX shows antibacterial activity against *E. faecalis* (15,16,17). In accordance with other studies, no significant increase in the OD values of CHX and its combinations (except NaOCl + CHX) were observed in this recent study. However, NaOCl + CHX showed less antibacterial activity with increased OD values. This may be due to the orange-colored residue made of parachlorophenol (PCU) or chloropfenilguanidil-1,6-diguanidil-hexaze (PCGH), which can be obtained when CHX and NaOCl are combined (18, 19). These residues may have had negative effects on the interaction between the root filling and the canal wall dentin by blocking dentin tubules (18, 20). In order to prevent these solution's interaction, it is recommended to irrigate the root canals with saline, sterile distilled water or alcohol before irrigation with CHX, and NaOCl left in the canal can be aspirated with a needle, dried with paper cones or ultrasonic activation with EDTA (20,21,22). However, in the present study, the recommended processes to prevent the interaction of these two solutions mentioned above were not used because of their variations in the experimental procedure. Another important issue noted with the NaOCl + CHX group is that the antibacterial activity did not decrease in the first 12 hours but did decrease after hour 12 (Figure 4). This may be because of the residue that accumulated on the root canal while taking the sample, and/or the possibility of PCU and PCGH being toxic to *E. faecalis* and the decrease observed in these effects at the end of hour 12.

je steriliziranih korijena (negativna kontrolna skupina). Tijekom razdoblja inkubacije bilo je jasno određeno u kojemu se razdoblju reprodukcija događala, usporila i nazadovala.

Poznato je da se antibakterijska aktivnost otopina za irigaciju povećava s povećanjem volumena i vremenom primjene irigacije (10, 11). U ovoj je studiji utvrđeno da standard irigacije uključuje vrijeme od ukupno 5 minuta s 5 mL otopine za sve kanale. U kombiniranim otopinama ukupni upotrijebljeni volumen bio je 2,5 mL + 2,5 mL za svaku otopinu. Za MTAD je proizvođač preporučio primjenu od 5 mL za svaki kanal. Zato je za MTAD također korišten jednaki interval volumena i vremena kao i za ostale otopine, čineći tako sve otopine u pokusu usporedivima. Svi se korijenski kanali također irigiraju u fazi nakon pokusa s 5 mL fiziološke otopine kako bi se postiglo maksimalno razrjeđivanje zaostale otopine. Na taj je način utjecaj prenesene otopine na medij sveden na minimum, a zaostali antibakterijski učinak je smanjen.

U ovom istraživanju korišten je redosljed primjene otopina koji su preporučili istraživači (12, 13). Potvrđeno je da je zaostali antimikrobni učinak još prisutan ako se aktivnost otopine ne neutralizira inaktivatorima (14). No kako bi se mogli standardizirati pokusi provedeni u ovom istraživanju, nije obavljena neutralizacija ispitanih otopina. Jedan od razloga jest i to što su neke ispitane otopine, poput MTAD-a i SC-a, zaštićeni proizvodi za koje nije poznato postoje li kemijski inaktivatori. Zato, kako bi se postigla jedinstvenost u metodologiji pokusa i olakšao proces za usporedbu razmjerno velikog broja otopina, nijedan testni uzorak nije bio inaktiviran.

Mnogi su istraživači istaknuli da CHX pokazuje antibakterijsko djelovanje na *E. faecalis* (15, 16, 17). U skladu s drugim studijama, u ovom nedavnom istraživanju nije uočeno značajno povećanje OG vrijednosti za CHX i njegove kombinacije (osim NaOCl + CHX). No NaOCl + CHX pokazao je manje antibakterijske aktivnosti s povećanim vrijednostima OG-a. To se može dogoditi zbog narančasto obojenih ostataka izgrađenih od paraklorofenola (PCU) ili kloropfenilguanidil-1,6-diguanidil-heksaza (PCGH) koji se mogu dobiti kada se kombiniraju CHX i NaOCl (18, 19). Ti ostatci mogu negativno utjecati na interakciju između korijenskog punjenja i dentinskih stijenki kanala te blokirati dentinske tubuluse (18, 20). Kako bi se spriječila interakcija tih otopina preporučuje se irigacija korijenskih kanala fiziološkom otopinom, sterilnom destiliranom vodom ili alkoholom prije irigacije CHX-om, a NaOCl koji je ostavljen u kanalu može se aspirirati iglom, osušiti papirnatim štapićima ili ultrazvučnom aktivacijom EDTA-e (20, 21, 22). No u ovoj studiji preporučeni postupci za sprječavanje interakcije spomenutih otopina nisu korišteni zbog njihovih varijacija u pokusnom postupku. Drugo što je važno, a zabilježeno je kod skupine NaOCl + CHX jest da se antibakterijska aktivnost nije smanjila u prvih 12 sati, nego nakon 12 sati (slika 4.). Razlog mogu biti ostatci koji su nakupljeni u korijenskom kanalu tijekom uzimanja uzorka i/ili mogućnosti da su PCU i PCGH toksični za *E. faecalis* te smanjenje opaženoga nakon 12 sati.

Kako nije utvrđeno povećanje OG vrijednosti u skupini EDTA + CHX tijekom vremena ($p > 0,05$), ta kombinacija može učinkovito antibakterijski djelovati na *E. faecalis*. Liu i

Since no increase was found in the EDTA + CHX group's OD values over time ($p > 0.05$), this combination has the potential to show effective antibacterial activity against *E. faecalis*. Liu et al. (23) reported that the combined use of CHX and EDTA had antibacterial activity and it was also better than MTAD and EDTA + NaOCl. The present study also supports these results. Gonzalez-Lopez et al. (24) observed a pink-colored residue when CHX and EDTA were mixed. In their study, Rasimick et al. (25) showed a white-colored salt when these two materials were mixed. Later, Prado et al. (21) examined the interaction of CHX and EDTA and observed a milky residue and concluded that this was a result of an acid-base reaction of the combination. The results of the present study confirmed that the white and/or salt residue formed by these two materials does not negatively affect the antibacterial activity of CHX in infected root canals.

No studies have been conducted so far on whether ClO_2 + CHX shows antibacterial activity against *E. faecalis*. According to the results of the present study, ClO_2 + CHX is effective in the elimination of *E. faecalis*. These two agents do not form any reactions nor do they negatively influence each other (26, 27) and they maintain their antibacterial activity at all times when used in combinations (Figure 4,6).

Although it has been reported in a great number of studies that MTAD has antibacterial activity against *E. faecalis* (23, 28), no studies have been found comparing the antibacterial characteristics of CHX + MTAD to MTAD. The results of this study showed that the CHX + MTAD and MTAD groups have similar antibacterial activities against *E. faecalis* ($p > 0.05$) (Figure 7). The CHX + MTAD combination did not cause any negative change in the antibacterial characteristics of either solution. This important characteristic can be evaluated with further studies not only on *E. faecalis* but also on the elimination of other bacteria that are also responsible for endodontic infections.

In this study, an increase was found in the OD value of the H_2O_2 + NaOCl group after 12 hours, and this increase was found to be different from the negative control group's ($p < 0.05$) (Figure 5,9). The combined use of these two solutions has been recommended to ease organic and inorganic debris from the root canal, and it has disinfecting and whitening properties through its foaming effect (29). The antibacterial activity of an irrigant increases as its volume increases (30). Since, NaOCl's volume in combined use is less than when it is used alone, a decrease in antibacterial activity is expected. The antibacterial activity of H_2O_2 was also found to be insufficient in the present study. Therefore, it was speculated that the combined use of these two solutions did not positively contribute to the solutions' antibacterial characteristics. Another reason for the decreased antibacterial activity may be due to decreased hydroxyl radicals, which actually creates antibacterial activity with the reaction of the two solutions (31).

In the EDTA + NaOCl and SC + NaOCl groups, a time-dependent increase was found in measurements after hour 6 (Figure 5). When compared to the negative control group, the antibacterial activity in the EDTA + NaOCl group was found to be less than the activity in the SC + NaOCl group. The reason for the decrease in antibacterial activity may be

suradnici (23) pokazali su da kombinirana primjena CHX-a i EDTA-e ima antibakterijsko djelovanje, te da je također uspješnija od kombinacije MTAD-a i EDTA + NaOCl. Ova studija također podupire te rezultate. Gonzalez-Lopez i suradnici (24) uočili su ostatak ružičaste boje kada su se pomiješali CHX i EDTA-u. U svojoj studiji su Rasimick i suradnici (25) istaknuli da su dobili sol bijele boje kada su miješali ta dva materijala. Poslije su Prado i suradnici (21) ispitali interakciju CHX-a i EDTA-e i uočili mliječni ostatak te zaključili da je to rezultat kiselo-bazne reakcije kombinacije. Rezultati ove studije potvrdili su da bijeli ostatak i/ili sol stvorena s tim dvama materijalima ne utječe negativno na antibakterijsko djelovanje CHX-a u zaraženim korijenskim kanalima.

Do sada nisu provedena ispitivanja djeluje li ClO_2 + CHX antibakterijski na *E. faecalis*. Prema rezultatima ove studije, ClO_2 + CHX učinkovito uklanja tu bakteriju. Ta dva sredstva ne stvaraju nikakvu reakciju, niti negativno utječu jedno na drugo (26, 27) i zadržavaju svoje antibakterijsko djelovanje u svakom trenutku kada se koriste u kombinacijama (slika 4., 6.).

Iako je u mnogim studijama objavljeno da MTAD antibakterijski djeluje na *E. faecalis* (23, 28), nisu pronađena istraživanja u kojima su autori uspoređivali antibakterijska svojstva CHX + MTAD-a i MTAD-a. Rezultati ove studije pokazali su da skupine CHX + MTAD i MTAD imaju slične antibakterijske aktivnosti kad je riječ o *E. faecalis* ($p > 0,05$) (slika 7.). Kombinacija CHX + MTAD nije uzrokovala nikakve negativne promjene u antibakterijskim karakteristikama ni u jednoj otopini. Ovo važno svojstvo može se procijeniti u daljnjim istraživanjima ne samo na *E. faecalis*, nego i u uklanjanju drugih bakterija koje su također odgovorne za endodontske infekcije.

U ovom istraživanju prikazano je povećanje vrijednosti u skupini OG H_2O_2 + NaOCl nakon 12 sati, a utvrđeno je da se taj porast razlikuje od onoga u negativnoj kontrolnoj skupini ($p < 0,05$) (slike 5., 9.). Preporučuje se kombinirana upotreba tih dviju otopina za ublažavanje organskih i anorganskih nečistoća iz korijenskog kanala, a kako se pjene, imaju svojstva dezinfekcije i izbjeljivanja (29). Antibakterijsko djelovanje irigansa povećava se s povećanjem volumena (30). Budući da je volumen natrijeva hipoklorita u kombiniranoj uporabi manji negoli kada se koristi sam, očekuje se smanjenje antibakterijske aktivnosti. U ovoj studiji također je utvrđeno nedovoljno antibakterijsko djelovanje H_2O_2 . Zato se nagađalo da kombinirana primjena tih dviju otopina ne pridonosi pozitivno antibakterijskim svojstvima otopina. Drugi razlog za smanjene antibakterijske aktivnosti može biti smanjenje hidroksilnih radikala, što zapravo stvara antibakterijsko djelovanje reakcijom dviju otopina (31).

U skupinama EDTA + NaOCl i SC + NaOCl utvrđeno je vremenski ovisno povećanje u mjerenjima nakon 6 sati (slika 5.). U usporedbi s negativnom kontrolnom skupinom, istaknuto je da je antibakterijska aktivnost u skupini EDTA + NaOCl manja od one u skupini SC + NaOCl. Razlog za to može biti negativni učinak EDTA-e na tkivno otapanje natrijeva hipoklorita i smanjenje količine aktivnoga klora u kombinaciji (32).

related to EDTA's negative effect on the tissue-dissolving capacity of NaOCl and decrease in the amount of active chlorine in the combination (32).

It has previously been reported that NaOCl + MTAD is successful in *E. faecalis* elimination (33). Tay et al. (34) reported a brown liquid developed when NaOCl and MTAD were combined and that this combination decreased the dentine substantivity of MTAD (35). For these reasons, it has been reported that the canals need to be irrigated in intervals with saline. In the present study, no decrease was found in the combination's antibacterial activity. Thus, the results of the current study support Shabahang and Torabinejad's study (13), which states that the antibacterial effect of the combination of 1.3% NaOCl and MTAD is an effective solution in eradicating *E. faecalis*.

ClO_2 is a strong oxidizing agent, and it effectively kills pathogenic microorganisms (36). ClO_2 actually has smear layer removing characteristics and the capacity to dissolve organic tissue (37-42). In this study, it was found that when used alone and in combination with other solutions, ClO_2 showed similar antibacterial characteristics with NaOCl and its combinations against *E. faecalis*. However, ClO_2 and the SC + ClO_2 , EDTA + ClO_2 , H_2O_2 + ClO_2 and ClO_2 + CHX combinations showed relatively higher antibacterial characteristics compared to the NaOCl combinations (Figure 6). These results are parallel to Eddy et al.'s findings (37), which stated 10 % and 13.8 % Chlorine dioxide and 5.25 % NaOCl were both effective in eliminating *E. faecalis* from the dental disks within 30 min.

According to the results of the present study, a fair amount of increase was found in the OD values of the EDTA + ClO_2 and SC + ClO_2 groups; however, this increase was found to be statistically similar to the negative control group ($p > 0.05$) (Figure 6). This increase in the OD levels of the EDTA + ClO_2 group may be due to the decrease in antibacterial activity as a result of the ClO_2 -oxidizing effect against EDTA (26). In addition, this increase in the OD values of the samples treated with SC+ ClO_2 was found to be lower than that of EDTA + ClO_2 (Figure 6). This may be due to the antibacterial characteristic of the surface-active agent (cetrimide) in SC (43). Since there are no studies researching the antibacterial activity of SC + ClO_2 combinations, further studies are needed to explain the reasons for this increase.

There was a continuous increase in the OD values of the SC + ClO_2 and SC + NaOCl groups in the OD measurements after hour 6 (Figure 8). While the SC + ClO_2 group's increase was found to be significantly similar to the negative control group's ($p > 0.05$) at all hours (Figure 8), the measurements at hours 30, 36 and 48 in the SC + NaOCl group were found to be significantly higher than those of the negative control group's ($p < 0.05$) (Figure 8). Thus, SC + ClO_2 showed better antibacterial activity than SC + NaOCl. This may be lower inhibitory effect of SC on ClO_2 than the inhibitory effect of NaOCl.

A time-dependent increase was found in the EDTA + H_2O_2 group. Although the increase in the OD values of EDTA + H_2O_2 within the first 18 hours was significantly similar to the positive control group ($p > 0.05$), it was significant-

Prije je objavljeno da je NaOCl + MTAD uspješan u uklanjanju *E. faecalis* (33). Tay i suradnici (34) ističu da je smeđa tekućina nastala kada su kombinirani NaOCl i MTAD te da je ta kombinacija smanjila dentinsku vezu MTAD-a (35). Iz tih je razloga objavljeno da se kanali trebaju u intervalima irigirati fiziološkom otopinom. U ovoj studiji nije uočeno smanjenje antibakterijskog djelovanja te kombinacije. Dakle, rezultati ove studije podupiru Shabahangovu i Torabinejadovu studiju (13) u kojoj se ističe da je antibakterijski učinak kombinacije 1,3-postotnoga natrijeva hipoklorida i MTAD-a učinkovito rješenje u iskorjenjivanju *E. faecalis*.

ClO_2 snažno je oksidacijsko sredstvo i učinkovito ubija patogene mikroorganizme (36). Zapravo ima svojstva uklanjanja zaostanog sloja i otapanja organskoga tkiva (37 – 42). U ovoj studiji ustanovljeno je da, kada se koristi sam i u kombinaciji s drugim otopinama, ClO_2 ima slična antibakterijska svojstva kao i NaOCl i njegove kombinacije kad je riječ o *E. faecalis*. No kombinacije ClO_2 i SC + ClO_2 , EDTA + ClO_2 , H_2O_2 + ClO_2 i ClO_2 + CHX pokazale su razmjerno bolja antibakterijska svojstva u usporedbi s kombinacijama u kojima je NaOCl (slika 6.). Ti su rezultati jednaki kao i u izvješćima Eddyja i ostalih (37) koji su izjavili da su 10-postotni i 13,8-postotni klorov dioksid te 5,25-postotni NaOCl djelotvorni u uklanjanju *E. faecalis* iz dentinskih diskova u roku od 30 minuta.

Prema rezultatima dobivenima u ovoj studiji, ustanovljen je priličan porast vrijednosti OG-a u skupinama EDTA + ClO_2 i SC + ClO_2 , no ustanovljeno je da je taj porast statistički sličan negativnoj kontrolnoj skupini ($p > 0,05$) (slika 6.). To povećanje razine OG-a u skupini EDTA + ClO_2 može biti posljedica smanjenja antibakterijske aktivnosti kao rezultat učinka oksidacije ClO_2 na EDTA-u (26). Uz to, utvrđeno je da je to povećanje OG vrijednosti uzoraka tretiranih s otopinom SC + ClO_2 niže od onih tretiranih otopinom EDTA + ClO_2 (slika 6.). To može biti posljedica antibakterijskih svojstava površinski aktivnoga agensa (cetrimida) u SC-u (43). Budući da ne postoje studije u kojima je istraženo antibakterijsko djelovanje kombinacija SC + ClO_2 , potrebne su dodatna istraživanja za objašnjenje toga porasta.

Zabilježeno je kontinuirano povećanje OG vrijednosti u skupinama SC + ClO_2 i SC + NaOCl u OG mjerenjima nakon 6 sati (slika 8.). Dok je uočeno da je porast u skupini SC + ClO_2 vrlo sličan negativnoj kontrolnoj skupini ($p > 0,05$) u svim satima (slika 8.), rezultati mjerenja poslije 30, 36 i 48 sati u skupini SC + NaOCl bili su značajno veći od onih u negativnoj kontrolnoj skupini ($p < 0,05$) (slika 8.). Zato je kombiniranje SC + ClO_2 pokazalo bolje antibakterijsko djelovanje od onoga u skupini SC + NaOCl. To može biti posljedica nižega inhibicijskog učinka SC-a na ClO_2 od inhibicijskog učinka natrijeva hipoklorita.

Nadalje, utvrđeno je povećanje ovisno o vremenu u skupini EDTA + H_2O_2 . Iako je porast OG vrijednosti EDTA + H_2O_2 u prvih 18 sati bio sličan pozitivnoj kontrolnoj skupini ($p > 0,05$), značajno se razlikovao od pozitivne kontrolne skupine nakon 30 sati ($p < 0,05$) (slika 9.). Razlog za porast u toj skupini može biti medij stvoren kombinacijom tih otopina jer je bio pogodan za rast *E. faecalis*. No potrebne su dodatne studije za potvrdu tih rezultata.

ly different from the positive control group after hour 30 ($p < 0.05$) (Figure 9). The reason for the increase in this group may be because the medium created with the combination of these solutions was a suitable medium for *E. faecalis* to grow. However, further studies are needed to confirm these results.

Conclusions

Within the limitations of this study, the following conclusions can be drawn:

EDTA is not a suitable solution for providing antibacterial activity in the irrigation process of root canals. Although it did not have any negative effects in combinations with CHX and MTAD, combined uses of EDTA and H_2O_2 solutions may be harmful for disinfecting the root canals since they can potentially provide a medium for bacteria reproduction; Chelation agents, such as EDTA and SC, can be used for the removal of the smear layer from the root canal walls. However, their application together with NaOCl reduces the antibacterial effect of these agents. For these reasons, additional irrigation with another irrigation solution and/or solution combination may be suggested; ClO_2 is chemically similar to NaOCl and when used in combination with H_2O_2 , CHX, EDTA or SC, its antibacterial activities are less affected than with combinations of NaOCl. Therefore, ClO_2 , with its positive properties, may be considered as an alternative to NaOCl and suitable for routine clinical use; Overall it can be concluded that the SC + CHX combinations can be recommended as the most effective irrigation regimen against *E. faecalis* in persistent endodontic infections.

Conflict of Interest

The authors report no conflict of interest

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Contribution to the paper

H.B.O. Carried out the study experiments, collected the data, wrote and edited the manuscript; **F.K.C.** Contributed to experimental design of the study, helped with analysis of the data, interpreted and discussed the results; **Z.S.** Contributed to experimental design of microbiological part of the study, commented on the manuscript at the microbiological stages; **F.O.** Contributed to research idea, commented on the manuscript at all stages, critically proof read the manuscript and gave final approval for publication.

Zaključci

Uz ograničenja u ovoj studiji mogu se izvući sljedeći zaključci:

EDTA nije prikladna otopina za postizanje antibakterijske aktivnosti pri irigaciji korijenskih kanala. Iako nije imala negativnih učinaka u kombinaciji s CHX-om i MTAD-om, kombinirana uporaba otopina EDTA i H_2O_2 može štetiti u dezinfekciji korijenskih kanala zato što mogu stvoriti medij za razmnožavanje bakterija; Sredstva za kelaciju, poput EDTA-e i SC-a, mogu se upotrijebiti za uklanjanje zaostatnog sloja sa zidova korijenskog kanala. No njihova primjena zajedno s natrijevim hipokloritom smanjuje njihov antibakterijski učinak. Zato se može predložiti dodatna irigacija drugom otopinom i/ili kombinacija otopina; ClO_2 je kemijski sličan natrijevu hipokloritu i kada se koristi u kombinaciji s H_2O_2 , CHX-om, EDTA-om ili SC-om, a njihovo antibakterijsko djelovanje manje je negoli kod kombinacija s natrijevim hipokloritom. Zato se ClO_2 , sa svojim pozitivnim svojstvima, može smatrati alternativom natrijevu hipokloritu i prikladnim za rutinsku kliničku upotrebu; Sveukupno se može zaključiti da se kombinacija SC + CHX može preporučiti kao najučinkovitiji protokol za irigaciju u slučaju *E. faecalis* kod perzistentnih endodontskih infekcija.

Sukob interesa

Autori ističu da nisu bili u sukobu interesa.

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H. B. O. – obavljala je eksperimente, prikupljala podatke te napisala i uredila rukopis; **F. K. C.** – pridonijela je eksperimentalnom oblikovanju studije, pomagala je pri analizi podataka, interpretirala i raspravljala o rezultatima; **Z. S.** – pridonio je eksperimentalnom oblikovanju mikrobiološkog dijela studije, komentirao rukopis u mikrobiološkoj fazi; **F. O.** – suradujući u istraživačkoj ideji komentirao je rukopis u svim fazama, kritički je pročitao rukopis i dao konačno odobrenje za objavljivanje.

Sažetak

Ciljevi: U ovoj studiji istraživalo se antibakterijsko djelovanje pojedinačne i kombinirane primjene otopina za irigaciju u slučaju kontaminacije bakterijom *Enterococcus faecalis*. i to 5,25-postotnog natrijeva hipoklorita (NaOCl), 2-postotnog klorheksidina (CHX), 17-postotne etilendiamintetraočne kiseline (EDTA), 3-postotnog vodikova peroksida (H₂O₂), MTAD-a, SmearCleara (SC) i 13,8-postotnog klorova dioksida (ClO₂). **Materijal i metode:** Nasumično je grupirano 280 jednokorijskih ljudskih pretkutnjaka (premolara) u 26 testnih skupina i u 2 kontrolne (negativna i pozitivna) te su 24 sata inkubirane bakterijom *E. faecalis*, osim negativne kontrolne skupine. Testirani rezultati su sljedeći: NaOCl; CHX; ClO₂; MTAD; SC; EDTA; H₂O₂; NaOCl + CHX; NaOCl + MTAD; SC + NaOCl; EDTA + NaOCl; H₂O₂ + NaOCl; ClO₂ + CHX; CHX + MTAD; SC + CHX; EDTA + CHX; CHX + H₂O₂; ClO₂ + MTAD; SC + ClO₂; EDTA + ClO₂; ClO₂ + H₂O₂; SC+MTAD; EDTA+MTAD; MTAD + H₂O₂; SC + H₂O₂; i EDTA + H₂O₂. Zabilježene vrijednosti optičke gustoće su 0, 6, 12, 18, 24, 30, 36, 42 i 48 h, a za svaku otopinu stvorena je krivulja rasta bakterija. **Rezultati:** CHX, MTAD i ClO₂ pokazali su visok potencijal smanjenja *E. faecalis* samostalno i u svim kombinacijama. U skupinama EDTA, H₂O₂, H₂O₂ + EDTA, H₂O₂ + NaOCl i SC + NaOCl postignuto je manje antibakterijsko djelovanje od onoga u ostalima. U skupini SC + CHX zabilježen je najbolji antibakterijski učinak na *E. faecalis*. **Zaključak:** Kombinacija SC + CHX može se preporučiti kao najučinkovitiji protokol za irigaciju u slučaju dugotrajnih endodontskih infekcija s *E. faecalis*.

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Ključne riječi

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