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IZVORNI ZNANSTVENI RAD
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Uklanjanje biofilma s bakterijom *Enterococcus faecalis* iz korijenskog kanala pomoću pasivnog ultrazvučnog ispiranja i sustava *RinsEndo*

Eradication of Intracanal Enterococcus Faecalis Biofilm by Passive Ultrasonic Irrigation and RinsEndo System

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Sažetak

Svrha: Usporediti antimikrobno djelovanje triju tehnika ispiranja korijenskog kanala s obzirom na jednako vrijeme ispiranja i jednak volumen sredstva za ispiranje. **Ispitanici i metode: Četrdeset i osam jednokorijenskih zuba inokulirano je** 48 sati suspenzijom *Enterococcus faecalisa.* Preostalih šest uzoraka korišteno je za negativnu kontrolu. Trideset i šest kanala nasumično je raspoređeno u tri eksperimentalne skupine: 1. grupa: konvencionalno ispiranje brizgalicom i iglom; 2. grupa: automatsko-dinamično ispiranje (RinsEndo); 3. grupa: pasivno ultrazvučno ispiranje (PUI). U prvom protokolu korišteno je 20 ml 3-postotnoga natrijeva hipoklorita, a u drugom protokolu kanali su isprani istim tehnikama i istom otopinom tijekom 45 sekundi. Uzorci iz korijenskih kanala kultivirani su, te su izbrojene bakterijske kolonije. **Rezultati:** U protokolu standardiziranog volumena irigansa, sistem *RinsEndo* bio je učinkovitiji od *PUI-ja* ($p < 0,01$). U protokolu standardiziranog vremena ispiranja nije bilo značajnih razlika izmedu tehnika ispiranja ($p > 0,05$). U skupini *RinsEndo* pronađen je najveći broj uzoraka s minimalnim brojem bakterija *E. faecalis*. **Zaključak:** Pri ispiranju korijenskih kanala istim volumenom irigansa, sistem *RinsEndo* učinkovitiji je od *PUI-ja*. *RinsEndom* postignuto je i najveće smanjenje bakterija u oba protokola, koristeći se najmanjom količinom irigansa te ostvarujući najdulje vrijeme djelovanja irigansa u kanalu.

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Ključne riječi
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Uvod

Uspjeh endodontskog liječenja zuba ovisi o uklanjanju vitalnoga ili nekrotičnog pulpnog tkiva iz složenoga kanalnog sustava. Ispiranje korijenskog kanala važan je dio njegove biomehaničke obrade s obzirom na to da, nakon samo mehaničke instrumentacije, od 35 do 40 posto površine kanala ostaje neočišćeno (1). Štoviše, dokazano je da nakon strojne instrumentacije korijenskog kanala ostaju neočišćene i inficirane intrakanalne nepravilnosti kao što su istmusi, ramifikacije i proširenja (2). Nadalje, prisutnost mikroorganizama i debrisa u tim nedostupnim dijelovima kanalnoga sustava može spriječiti kvalitetno brtvljenje materijalom za punjenje (3) i uzrokovati stalno mikrobno curenje kroz korijenski kanal i perapikalnu upalu.

Natrijev hipoklorit (NaOCl) najpopularnije je i najistraženije sredstvo za ispiranje korijenskog kanala (4). No istraživanja su pokazala da konvencionalno ispiranje korijenskog

Introduction

The success of the endodontic treatment depends on the removal of vital or necrotic pulp tissue and minimizing the amount of pathological debris from the complex root canal system. Root canal irrigation seems to be undeniably important during biomechanical preparation, particularly because, during mechanical instrumentation, approximately 35-40% of the root canal surface remained unchanged (1). Moreover, it has been revealed that even after the use of Ni-Ti rotary instruments, root canal imperfections such as isthmuses, ramifications and fins, remained untouched and infected (2). Furthermore, a continuous presence of microorganisms, their by-products and debris in these unreached areas may prevent close adaptation and penetration of the sealer (3) resulting in the persistent microbial leakage through the root canal, and periradicular inflammation.

kanala različitim koncentracijama NaOCl-a ne može potpuno ukloniti mikroorganizme iz toga dijela zuba (5). Najveći je problem složenost kanalnoga sustava zuba, što onemogućuje blizak kontakt sredstva za ispiranje sa stijenkama korijenskog kanala, a to je potrebno za učinkovito djelovanje antimikrobnog sredstva (6). Nadalje, dokazano je da sredstvo za ispiranje dopire do 1 mm od vrha igle (7), a s obzirom na to da se igla obično postavlja u koronarnu ili srednju trećinu kanala (8) upitno je antimikrobno djelovanje irigansa u apikalnoj trećini (7). Zbog toga se istražuju novi načini ispiranja korijenskog kanala koji bi omogućili prodiranje irigansa u apikalnu trećinu.

Iako je primjena ultrazvučnih uređaja u endodonciji počela potkraj 50-ih godina 20. stoljeća, prednost ultrazvučne energije u dezinfekciji korijenskog kanala prvi su put opisali Weller i suradnici (9) 1980. godine. Tijekom pasivnoga ultrazvučnog ispiranja (PUI) energija se prenosi s oscilirajućeg nastavka na sredstvo za ispiranje, uzrokujući stvaranje ultrazvučnih valova u tekućini (10). Pasivno ultrazvučno ispiranje pokazalo se učinkovitim u uklanjanju zaostatnog sloja i debrisa (11) te intrakanalnog biofilma (12). Sistem *RinsEndo*® (Dürr Dental GmbH & Co., Bietigheim-Bissingen, Njemačka) automatski je sustav čije se djelovanje temelji na tehnologiji pritiska i povlačenja te na aktivaciji sredstva za ispiranje u korijenskom kanalu (1,5 Hz). *RinsEndo*® pokazao se učinkovitijim od konvencionalnog ispiranja iglom i brizgalicom jer irigans dublje prodire u dentin (13) i tako smanjuje broj bakterija (14). Dosadašnja istraživanja usporedbe *RinsEndoa* i *PUI-ja* dala su kontradiktorne rezultate (15, 16).

Svrha ovog istraživanja bila je usporediti antimikrobno djelovanje ispiranja iglom i brizgalicom, sistemom *RinsEndo* i *PUI-jem* na biofilm s bakterijom *Enterococcus faecalis*, kad se koristimo standardiziranim volumenom natrijeva hipoklorita ili standardiziranim vremenom ispiranja.

Materijal i metode

Izbor i priprema uzoraka

Uzorak se sastojao od 60 zuba izabranih među 167 izvađenih humanih jednokorijenskih zuba na temelju kriterija sličnog volumena korijenskog kanala koji je izmjeren nakon ProTaper-tehnike instrumentacije. Protokol istraživanja odobrilo je Etičko povjerenstvo Stomatološkog fakulteta Sveučilišta u Zagrebu. Svi zubi izvađeni su zbog parodontnih razloga. Zubi s karijesom korijena i endodontski liječeni zubi isključeni su iz istraživanja. Svi zubi bili su intaktni i s razvijenim korijenom.

Nakon čišćenja površine korijena parodontnom kiretom, 167 zuba uronjeno je jedan sat u 5-postotnu otopinu natrijeva hipoklorita (NaOCl) kako bi se s površine uklonio organski materijal. Kruna zuba uklonjena je dijamantnim fisurnim svrdlom # 016 (Komet, Rock Hill, SC, SAD) uz vodeno hlađenje. Radna duljina bila je postavljena na 12 mm – 1 mm kraće od duljine koja je određena uvođenjem K-file instrumenta veličine 10 ili 15 (Dentsply Maillefer, Ballaigues, Švi-

Sodium hypochlorite (NaOCl) is currently the most popular and most investigated irrigant (4). However, it has been reported that conventional syringe irrigation using various concentrations of NaOCl cannot completely eliminate microorganisms from the root canal system (5). The main problem is the complexity of the root canal space that hinders direct contact of the irrigant with internal root surfaces, which is necessary for effective action of antimicrobial irrigants (6). Moreover, it has been shown that an irrigant is delivered only 1 mm deeper than the tip of the needle (7), and the needle tip is usually located in the coronal or middle third of the canal (8), hence the antimicrobial efficacy of the irrigant is questionable in the apical region (7). Therefore, attention has been given to the construction and investigation of new irrigation devices that would deliver irrigant to all intracanal areas.

Although the use of ultrasonic devices in endodontics started in the late 50s, the advantage of ultrasonic energy in the root canal disinfection without simultaneous mechanical instrumentation was for the first time described in 1980 by Weller et al. (9) During passive ultrasonic irrigation (PUI), the energy is transmitted from an oscillating file to an irrigant in the root canal creating ultrasonic waves (10). The PUI has been reported to be efficient in the removal of intracanal smear layer and debris (11), and to facilitate the disruption of endodontic biofilms (12). The RinsEndo® (Dürr Dental GmbH & Co., Bietigheim-Bissingen, Germany) is an automated system which uses pressure-suction technology to deliver the irrigant solution in the root canal and activates it automatically (1.5 Hz). The RinsEndo® has been shown to be superior over conventional syringe/needle irrigation in terms of deeper penetration of an irrigant in dentine (13), and reduction of the number of bacteria (14). The comparison with PUI yielded contradictory results (15, 16).

The aim of the study was to compare the antimicrobial efficacy of the syringe irrigation, the RinsEndo system and the PUI against 15 days old Enterococcus faecalis biofilm when used with 20 ml of the NaOCl or during 45 s.

Materials and methods

Selection and preparation of specimen

From a total of 167 extracted human single-rooted and single canal teeth with completely developed roots, 60 roots were selected for the study according to the approximately similar root canal volume, which was measured after the ProTaper root canal instrumentation. The study protocol was approved by the Ethics Committee of the School of Dental Medicine, University of Zagreb. All teeth were extracted because of periodontal disease. The teeth with root caries and endodontically treated teeth were excluded from the study. All teeth were intact and had a complete root development.

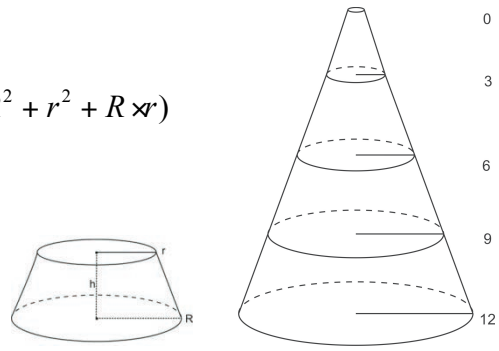
After cleaning the root surface with periodontal curettes, all 167 teeth were immersed in 5% sodium hypochlorite (NaOCl) for 1 hour to remove organic material from the root surface. The crowns were removed with a water-cooled diamond fissure bur # 016 (Komet, Rock Hill, SC, USA) and the working length (WL) was 12 mm, 1 mm shorter than the length established by introducing a K-file size 10 or 15

carska) u kanal te analizirana stereomikroskopom (Olympus SZX10, DF PL1.5, Hamburg, Njemačka). Dobiveni korijeni uloženi su u blokove od akrilatne smole (Polirepar S, poliDent, grad, Slovenija) (slika 1). Svi kanali instrumentirani su konvencionalnom sekvencijom rotacijske ProTaper Universal NiTi-tehnike (Dentsply/Maillefer, Tulsa, OK, SAD). Koronarna trećina kanala obrađena je instrumentima SX, a zatim preostali dio kanala instrumentima S1, S2, F1, F2 i F3 do radne duljine. Brzina rotacije bila je 300 rpm. Svaki kanal ispran je prije instrumentacije i između svakoga instrumenta s 1 ml 3-postotnoga NaOCl-a s pomoću brizgalice i (30-gauge) igle veličine 30. Nakon instrumentacije kanali su dvije minute ispirani s 2 ml 15-postotne etilendiaminotetraoctene kiseline (EDTA) (pH 7,7) kako bi se uklonio zaostatni sloj. Nakon toga svaki je kanal ispran s 2 ml 3-postotnoga NaOCl-a i 2 ml fiziološke otopine. Na kraju, kanali su posušeni sterilnim papirnatim štapićima veličine #F3 (ProTaper Universal, Dentsply/Maillefer, Tulsa, OK, SAD). Dno akrilatnog bloka premazano je trima slojevima laka kako bi se spriječilo curenje bakterija.

Određivanje volumena korijenskih kanala

Volumen korijenskih kanala izmjeren je u Laboratoriju za točna mjerenja Fakulteta strojarstva i brodogradnje Sveučilišta u Zagrebu. Promjer korijenskih kanala mjeren je na razmacima od 3, 6, 9 i 12 mm od radiografskog apeksa s pomoću rendgenskih snimaka u mesiodistalom i bukolingvalnom smjeru. Za mjerenje je korišten Profil projector (PJ-300H, Mitutoyo, Japan) postavljen na povećanje od 10 puta. Sva mjerenja obavio je jedan istraživač, a standardna pogreška određena je nakon triju mjerenja istog kanala. Volumen kanala izračunat je jednadžbom krnjega stošca:

$$V = \frac{\pi \cdot h}{3}(R^2 + r^2 + R \cdot r)$$



Budući da smo za svaki polumjer imali dvije vrijednosti (presjek kanala nema oblik kružnice, nego elipse), aproksimativno smo ga izračunali koristeći se formulama za izračunavanje površine kružnice $P = r^2 \cdot \pi$ i površine elipse $P_e = a \cdot b \cdot \pi \rightarrow r^2 \approx a \cdot b$. Kako se kanal sastoji od četiriju krnjih stožaca visine 3 mm, h = 3, volumene smo izračunali formulom:

(Dentsply Maillefer, Ballaigues, Switzerland) into the canal until it was visible at the apical foramen through a stereoscopic microscope (Olympus SZX10, DF PL1.5, Hamburg, Germany). Subsequently, roots were mounted vertically in blocks made of autopolymerized acrylic resin (Polirepar S, poliDent, Grad, Slovenia) and rubber frame (Figure 1). All root canals were prepared with the conventional sequence of ProTaper Universal NiTi rotary (Dentsply/Maillefer, Tulsa, OK, USA). The coronal two thirds of canals were prepared with shaping files SX and S1. Subsequently, rotary instrumentation was carried out using S1, S2, F1, F2 and F3 to WL. Rotation speed was set at 300 rpm. Each canal was irrigated before instrumentation and between files with 1 mL of 3% NaOCl using a disposable syringe and 30-gauge needle. After instrumentation, canals were rinsed with 2 ml 15% ethylenediaminetetraacetic acid (EDTA) (pH 7.7) for 2 min to remove the smear layer. After that, each canal was rinsed with 2 mL of 3% NaOCl and 2 ml saline solution. Finally, the canals were dried with sterile paper points, size#F3 (ProTaper Universal, Dentsply/Maillefer, Tulsa, OK, USA). The bottom of each acrylic block with inserted tooth was sealed with 3 layers of varnish to prevent leaking of bacteria from the root canal.

Determining the volume of root canals

The volume of root canals was calculated in the Laboratory for Accurate Length Measurements at the Faculty of Mechanical Engineering and Naval Architecture, University of Zagreb. Firstly, the diameter of each root canal was measured at the distances of 3, 6, 9 and 12 mm from the radiographic apex using radiographs in mesiodistal and buccolingual directions and Profil projector (PJ-300H, Mitutoyo, Japan) at 10x magnification. One investigator performed all measuring and the standard error was determined after 3 measurements of each root canal. The volume of each root canal was approximatively calculated using the equation for the truncated cone:

Because the cross section of root canal is elliptic, there were two values for each radius and it was approximatively calculated using formulas for circle area $P = r^2 \cdot \pi$ and ellipse area $P_e = a \cdot b \cdot \pi \rightarrow r^2 \approx a \cdot b$. Since the root canal is composed of 4 truncated cones with the height of 3 mm each, the volume of root canal was calculated according to the following equation:

$$V = \pi \cdot (r_{12}^2 + r_9^2 + r_{12} \cdot r_9) + \pi \cdot (r_9^2 + r_6^2 + r_9 \cdot r_6) + \pi \cdot (r_6^2 + r_3^2 + r_6 \cdot r_3) + \pi \cdot (r_3^2 + r_0^2 + r_3 \cdot r_0)$$

Kontrola sterilizacije

Uzorci su sterilizirani u plazmi. S obzirom na to da takva sterilizacija ima ograničenja te uspjeh može biti upitan kad je riječ o predmetima složene strukture (17), obavljena je kontrola sterilizacije svih 60 uzoraka. Svaki korijen bio je pohranjen u sterilnu plastičnu bočicu. Korijenski kanali napunjeni su sterilnim bujonom (brain heart infusion, 211059, Becton Dickinson, NJ, SAD) s pomoću sterilne 1-milimetarske tuberkulinske brizgalice (Becton Dickinson, Plastipak, NJ, SAD) i igle. Bočice su zatvorene i inkubirane na 37 °C u 100-postotnoj vlažnosti. Nakon 48 sati kanali su napunjeni 0,85-postotnom sterilnom fiziološkom otopinom. Uzorci iz korijenskog kanala prikupljeni su jednominutnim umetanjem dvaju papirnatih štapića veličine #30 (VDW GmbH, München, Njemačka) do radne duljine. Papirnati štapići stavljeni su u epruvete s 100 µl sterilne 0,85-postotne fiziološke otopine agitirane 1 minutu u vortexu. Tada je 10 µl sadržaja nasađeno na krvne podloge sa 7-postotnom konjskom krvi (211037, Becton Dickinson, NJ, SAD) te 48 sati inkubirano na 37 °C. Sterilizacija je potvrđena kada na podlogama nije bio uočen rast bakterija.

Kultivacija bakterije *Enterococcus faecalis* i kontaminacija

Bakterijska suspenzija pripremljena je miješanjem čiste kulture *E. faecalis* ATCC 29212 uzgajane 24 sata na krvnom agaru sa 7 posto konjske krvi i 2 ml sterilne 0,85-postotne fiziološke otopine. Gustoća suspenzije od 0,5 McFarlanda izmjerena je denzitometrom (Densimat, BioMérieux, Marcy l'Etoile, Francuska).

Četrdeset i osam korjenova podijeljeno je u tri eksperimentalne skupine (n = 12) i dvije kontrole (n = 6). U korijenske kanale eksperimentalne skupine i pozitivne kontrole ubrizgano je 18 µl bakterijske suspenzije. Preostalih šest korijenskih kanala u negativnoj kontroli napunjeno je s 18 µl sterilnog bujona. Na dno bočice nanesen je 1 ml sterilnog hraništa koje je služilo kao kontrola mogućeg curenja *E. faecalis* kroz korijenski kanal. Ulazi korijenskih kanala zatvoreni su staklenom pločicom kako bi se spriječila njihova dehidracija. Kanali su bili 48 sati inkubirani na 37 °C.

Protokoli ispiranja

Antimikrobna učinkovitost tehnika ispiranja analizirana je u dvama protokolima, s obzirom na standardizirani volumen irigansa u prvom protokolu i na standardizirano vrijeme ispiranja u drugom protokolu.

U prvom protokolu za ispiranje je korišteno 20 ml 3-postotnog natrijeva hipoklorita u sve tri eksperimentalne skupine.

Grupa 1 (n =12)

Korijenski kanali ispirani su s 20 ml NaOCl-a 80 sekundi brizgalicom od 20 ml i (30-gauge) iglom veličine 30. Igla je bila postavljena u kanal 1 mm kraće od radne duljine jer je dokazano da sredstvo za ispiranje prodire 1 mm ispod vrha igle (7).

Grupa 2 (n = 12)

Korijenski kanali isprani su sistemom *RinsEndo* prema uputama proizvođača. Irigans je unesen i aktiviran RinsEn-

Sterilization control

The study samples were sterilized in plasma. Since plasma sterilization has limitations and the success can be questionable for items with a narrow internal diameter and complex structure (17), sterilization control was performed in all 60 samples. Each selected root was placed in a sterile plastic vial. Root canals were filled with sterile broth culture (brain heart infusion, 211059, Becton Dickinson, NJ, USA) by using a sterile 1-ml tuberculin syringe without overflowing (Becton Dickinson, Plastipak, NJ, USA) and a needle. The vials were closed and incubated at 37°C in 100% humidity. After 48 h, the canals were filled with sterile 0.85% saline solution, and samples were collected by sequential use of 3 paper points size#30 (VDW GmbH, Munich, Germany) to WL for 1 minute. Paper points were placed in tubes containing 100 µl sterile 0.85% saline solution and agitated in vortex for 1 minute, and then 10 µl was plated onto blood agar plates containing 7% horse blood (211037, Becton Dickinson, NJ, USA) and incubated at 37°C for 48 h. Sterilization was confirmed when there was no growth of bacteria on the agar plates.

Cultivation of *Enterococcus faecalis* and contamination

A suspension was prepared by mixing a pure culture of *E. faecalis* ATCC 29212, grown in blood agar plates containing 7% horse blood for 24 h, with sterile 2 ml 0.85% saline solution. The density of 0.5 McFarland was measured by the densitometer (Densimat, BioMérieux, Marcy l'Etoile, France).

Forty-eight roots were randomly divided into 3 experimental groups (n=12) and 2 control groups (n=6). The root canals of the experimental groups and positive control were infected with 18 µl of bacterial suspension. The remaining six root canals were filled with 18 µl of sterile broth to serve as negative controls. The bottom of each vial was also filled with 1 ml sterile broth to serve as a control for possible leaking of *E. faecalis* from the root canal. The orifices of all root canals were closed with glass cover to prevent dehydration of the canals. The samples were incubated at 37°C for 24h.

Irrigation protocols

The antimicrobial efficacy of the three irrigation techniques was evaluated with respect to the standardized volume of the irrigant in the first protocol, and with regard to the standardized irrigation time in the second protocol.

In the first study protocol, in all three experimental groups three different irrigation techniques were performed with 20 ml of 3% NaOCl, by one examiner.

Group 1 (n=12)

The root canals were irrigated using a 20 ml syringe and a 30-gauge needle for approximately 80s. The needle was inserted to 1 mm short of the WL because it was shown that the irrigant penetrated only 1 mm deeper than the tip (7).

Group 2 (n=12)

The protocol for irrigation was carried out according to the manufacturer's instructions for RinsEndo. The irrigant

dom postavljenim na 6,2 ml min^{-1} rezultirajući totalnim vremenom ispiranja od 3,2 minute. Koristila se brizgalica od 20 ml kao spremnik za 20 ml NaOCl-a. Komprimirani zrak postavljen je na 4 bara (2,3 – 4,2 bara).

Grupa 3 (n = 12)

Ispiranje je obavljeno ultrazvučnim uređajem (Piezon Master 400; EMS, Nyon, Švicarska) i K-proširivačem veličine #15 (Endosonore; Maillefer, Ballaigues, Švicarska). U kanal je 30 sekundi kontinuirano unošeno 20 ml 3-postotnoga NaOCl-a. Tijekom *PUI-ja*, ultrazvučni instrument bio je postavljen u kanal 1 mm od radne duljine osiguravajući tako slobodnu oscilaciju instrumenta i slobodan tijek iriganса iz kanala.

Pozitivna kontrola (n = 6)

Korijenski kanali isprani su s 20 ml 0,85-postotne sterilne fiziološke otopine s pomoću igle veličine 30 postavljene 1 mm od radne duljine.

U drugom protokolu analizirano je antimikrobno djelovanje tehnika ispiranja u odnosu na vrijeme ispiranja od 45 sekundi za sve skupine. Trideset i šest uzoraka iz prvog istraživanja korišteno je i u drugom protokolu. Uzorci su sterilizirani u plazmi i nasumično podijeljeni u dvije kontrole (n = 6) i dvije eksperimentalne skupine s 12 uzoraka u svakoj. S obzirom na to da *PUI* može oštetiti površinu korijenskog kanala, za tu skupinu izabrano je 12 novih korjenova s volumenom od 5,77±1,08 mm^3 koji su zatim sterilizirani. Kontrola sterilizacije, inokulacija bakterijom *E. faecalis* i protokol ispiranja nakon 48 sati inkubacije bio je isti kao u prvom protokolu, osim što je vrijeme standardizirano na 45 sekundi. Volumen ubrizganog 3-postotnog NaOCl-a prikazan je u tablici 1.

was delivered and agitated by activation of the RinsEndo handpiece at a manufacturer's set rate of 6.2 ml min^{-1} resulting in an irrigation time of 3.2 min. The supplied 20ml syringe served as a reservoir for 20 ml of 3% NaOCl. The compressed air pressure supplying the handpiece was adjusted to 4 bars to ensure that it was within the recommended range (2.3 - 4.2 bar).

Group 3 (n=12)

The irrigation was performed with an ultrasonic device (Piezon Master 400; EMS, Nyon, Switzerland) and a stainless-steel K-type file size #15 (Endosonore; Maillefer, Ballaigues, Switzerland) with a medium power. 20 ml of 3% NaOCl was pumped during 30 seconds, with a continuous flush of the irrigant. During PUI, the ultrasonic file was introduced into the root canal to 1 mm short of the WL providing free oscillation of the file and free overflow of the irrigant from the canal.

Positive control (n=6)

Root canals were irrigated with 20ml of 0.85% sterile saline solution using syringe and 30-gauge needle, which was inserted to 1 mm short of the WL.

In the second study protocol, the antimicrobial efficacy of the irrigation techniques was analyzed with regard to irrigation time of 45 seconds for all groups. Thirty-six samples from the first protocol, except the samples from the PUI group, were used in the second protocol. They were sterilized in plasma and randomly selected into 2 controls (n=6) and 2 experimental groups of 12 samples each. Since PUI is the only one technique that might result in alteration of root canal shape and size, for PUI, new 12 roots with volume 5.77±1.08 mm^3 were randomly selected and sterilized. Sterilization control, *E. faecalis* inoculation and irrigation protocol after 24h of incubation were the same as in the first protocol except the irrigation time was set to 45s. The volume of delivered 3% NaOCl is presented in Table 1.

Tablica 1. Volumen 3% NaOCl-a i vrijeme ispiranja za eksperimentalne skupine i pozitivnu kontrolnu skupinu
Table 1 The volume of the 3% NaOCl and the irrigation time for experimental groups and positive control group

Irrigation technique	1st study protocol		2nd study protocol	
	• Volume (ml)	Irrigation time (sec)	Volume (ml)	Irrigation time (sec)
Syringe	20	80	11	45
RinsEndo	20	192	4.7	45
PUI*	20	30	30	45
Positive control	-	-	-	-

*passive ultrasonic irrigation

Prikupljanje uzoraka iz korijenskog kanala

Prvi uzorak uzet je iz pozitivne i negativne kontrole 48 sati nakon inokulacije bakterijom *E. faecalis*. Nakon ispiranja i prije uzimanja drugog uzorka, korijenski kanali u eksperimentalnim skupinama isprani su s 2 ml 3,86-postotnog natrijeva tiosulfata ($Na_2S_20_3$) kako bi se neutraliziralo antimikrobno djelovanje natrijeva hipoklorita (19).

Uzorci iz korijenskih kanala prikupljeni su nakon svakoga dezinfekcijskog protokola te u pozitivnoj i negativnoj

Microbial sampling of the canals

First samples were collected from positive and negative controls 24 h after inoculation of *E. faecalis*. Immediately after the irrigation and before second sample taking, root canals in the experimental groups were rinsed with 2 ml of 3.86% sodium thiosulfate ($Na_2S_20_3$) to neutralize the antimicrobial activity of NaOCl (19).

The root canals were sampled after each disinfection protocol both in the positive and the negative control group.

kontrolnoj skupini. Kanali su napunjeni s 10 µL sterilne 0,85-postotne fiziološke otopine. Nakon triju ciklusa aspiracije i potiskivanja sadržaja inzulinskom brizgalicom i iglom, sadržaj kanala prikupljen je i prenesen u Eppendorfovu epruvetu. Kolonije su prebrojene, a točan broj određen je prema faktoru razrjeđenja. Hedströmov instrument (Dentsply Maillefer) veličine 30 korišten je za struganje površine dentina čiji je debris također korišten u evaluaciji.

Hranilište s dna posudice također je nasađeno na krvnu podlogu i inkubirano 48 sati na 37 °C kako bi se potvrdilo da bakterije ne cure iz kanala.

Statistička analiza

Korišten je Kruskall-Wallisov test i Mann-Whitneyjev U-test za usporedbu rezultata između skupina. Razina značajnosti bila je postavljena na $p < 0,05$. Pojavnost negativnih nalaza kulture nakon protokola ispiranja između skupina određena je testom χ^2. Analize su određene s pomoću SPSS 11. 0 (Chicago, SAD).

Rezultati

Razmazi uzoraka bujona iz eksperimentalnih skupina i pozitivne kontrole nisu otkrili bakterije, čime je potvrđeno da nisu curile kroz kanal.

Nalazi negativne kontrole također nisu pokazali prisutnost bakterija.

Rezultati su pokazali značajnu razliku u broju kolonija *E. faecalis* nakon dezinfekcijskih tehnika i pozitivne kontrole ($p < 0,05$).

Tablica 2. pokazuje srednju vrijednost, medijan i raspon broja kolonija nakon ispiranja s 20 ml 3-postotnoga NaOCl-a. Nije bilo statistički značajne razlike između ispiranja brizgalicom i sistemom *RinsEndo* ($p > 0,05$). *RinsEndo* bio je učinkovitiji od *PUI-ja* ($p < 0,01$). Antimikrobna učinkovitost ispiranja brizgalicom i *PUI-jem* bila je slična ($p = 0,049$). Nakon dezinfekcije sistemom *RinsEndo*, četiri uzorka bila su

The canals were filled with 10 µL sterile 0.85% saline solution. After three aspiration-delivering cycles with a sterile insulin syringe, the canal content was aspirated and transferred to the Eppendorf tube. Colony forming units (CFUs) which had grown were counted and transformed into actual counts based on the dilution factor. A size 30 Hedström file (Dentsply Maillefer) was used to file vigorously the dentinal walls.

The broth samples from the bottom of the vial were also grown on blood agar plates and incubated for 48 hours at 37°C to check that there had not been any leakage of *E. faecalis* suspension from the root canal.

Statistical analysis

Intergroup comparisons were made by using nonparametric Kruskall-Wallis test and Mann-Whitney U-test. The significant level was set at $p<0.05$. The occurrence of negative cultures after irrigation protocols between the experimental groups was analyzed using the χ^2 test. Analyses were performed by using SPSS 11. 0 (Chicago, USA).

Results

Smears of the broth taken from the bottom of the vials of the experimental groups and positive controls did not show the presence of bacterial growth. The negative controls did not show the presence of bacteria.

There was a significant difference between the number of *E. faecalis* CFUs found after the disinfection techniques and in the positive control ($p<0.05$).

Table 2 reveals the mean, median and range of CFUs after the irrigation with 20 ml 3% NaOCl. There were no statistically significant differences between the syringe irrigation and the RinsEndo ($p>0.05$). The RinsEndo system was more effective than the PUI ($p<0.01$). The antimicrobial efficacy of the syringe irrigation and the PUI were similar ($p=0.049$). After the disinfection with the RinsEndo, there were four samples without the CFUs growth, and in the syringe/needle

Tablica 2. Broj kolonija bakterije *E. faecalis* nakon tri tehnike ispiranja i u pozitivnoj kontrolnoj skupini kod korištenja iste količine NaOCl-a
Table 2. Counts of *E. faecalis* CFUs after three irrigation techniques and for positive control group when using a defined amount of NaOCl

	Mean	Median	Range	SD‡
Syringe	3.96×10^1	1.90×10^1	0 to 2.00×10^2	5.84×10^1
RinsEndo	2.71×10^1	2.5	0 to 2.00×10^2	5.76×10^1
PUI*	8.98×10^1	8.00×10^1	8 to 2.00×10^2	7.44×10^1
Positive control	1.05×10^2	9.00×10^1	5.00×10^1 to 2.00×10^2	5.39×10^1

*passive ultrasonic irrigation
‡standard deviation

Tablica 3. Broj kolonija bakterije *E. faecalis* nakon tri tehnike ispiranja i u pozitivnoj kontrolnoj skupini u protokolu istog vremena ispiranja
Table 3 Counts of *E. faecalis* CFUs after three irrigation techniques and for positive control group when using a defined time of irrigation

	Mean	Median	Range	SD‡
Syringe	4.68×10^1	3.75×10^1	1.00×10^1 to 1.00×10^2	3.42×10^1
RinsEndo	3.24×10^1	4.0×10^1	1 to 5.00×10^1	1.72×10^1
PUI*	5.53×10^1	4.5×10^1	8 to 1.50×10^2	4.11×10^1
Positive control	1.31×10^2	1.29×10^2	7.00×10^1 to 2.00×10^2	4.96×10^1

*passive ultrasonic irrigation
‡standard deviation

bez rasta kolonija, a u skupini ispiranja brizgalicom i iglom, tri uzorka. U skupini *PUI-ja* i pozitivne kontrole nije bilo negativnih uzoraka.

Tablica 3. prikazuje srednju vrijednost, medijan i raspon broja kolonija nakon ispiranja uzoraka tijekom istoga razdoblja od 45 sekundi. Nije bilo statistički značajne razlike između testiranih tehnika ispiranja ($p > 0,05$) te ni u jednoj skupini nije bilo uzorka bez rasta kolonija.

Rasprava

Dosad je istraženo mnogo aktivnih tehnika ispiranja kako bi se poboljšalo djelovanje ispiranja u korijenskom kanalu (20). U ovom istraživanju analizirano je antimikrobno djelovanje konvencionalnog ispiranja brizgalicom i iglom, *PUI-ja* i sistema *RinsEndo* s obzirom na isto vrijeme ispiranja (45 s) i istu količinu NaOCl-a (20 ml).

Distribucija sredstva za ispiranje u korijenskom kanalu proporcionalno ovisi o njegovu volumenu; u velikim korijenskim kanalima turbulencije koje se stvaraju tijekom ispiranja mogu uzrokovati zaustavljanje debrisa i mikroorganizama (21). Zbog toga su u ovom istraživanju korišteni jednokorijenski zubi sličnog volumena kanala. *E. faecalis* korišten je kao reprezentativni endodontski patogen jer često preživljava kemomehaničku obradu korijenskog kanala, povezan je s primarnim i refraktornim endodontskim infekcijama (22) te se često upotrebljava u sličnim istraživanjima (18, 23). Vrijeme kolonizacije *E. faecalis* u dosadašnjim istraživanjima je različito (23). Dulje vrijeme kolonizacije bakterije stvara zreliji i klinički relevantniji biofilm, zbog čega se češće koristi u novijim istraživanjima (18, 24). U ovom istraživanju *E. faecalis* je inkubiran u kanalima 48 sati kao i u nekim prijašnjim radovima.

Dokazano je da učinkovitost ispiranja korijenskog kanala ovisi o volumenu sredstva za ispiranje (25) te o njegovoj koncentraciji i vremenu ispiranja (26). U ovom istraživanju najučinkovitije antimikrobno djelovanje uočeno je nakon ispiranja s 20 ml 3-postotnoga NaOCl-a sistemom *RinsEndo*, što je i potvrđeno najvećim brojem negativnih uzoraka (4 – 12). Prijašnja istraživanja također su potvrdila učinkovitost tog sistema, posebice u ispiranju apikalne trećine kanala (15, 16). Prema dosadašnjim istraživanjima, zvučno istraživanje i kavitacije u sredstvu za ispiranje tijekom *PUI-ja* pojačavaju učinkovitost irigansa (25, 27). Jedno od objašnjenja jest vrijeme ispiranja. Slaba učinkovitost *PUI-ja* u ovom istraživanju vjerojatno je posljedica kratkog vremena istraživanja – iznosilo je samo 20 sekundi, u usporedbi sa sistemom *RinsEndo* – 3,2 minute. Iako nema jasno definiranog vremena ispiranja potrebnoga za uklanjanje bakterija iz korijenskog kanala, vjerojatno je dulje djelovanje NaOCl-a ključno u eliminaciji bakterija *E. faecalis* (28). Druttman i Stock (29) također su zaključili da djelovanje i izmjena irigansa u korijenskom kanalu tijekom *PUI-ja* ovise više o vremenu nego o volumenu.

Rezultati ovog istraživanja pokazali su da 45-sekundno ispiranje 3-postotnim natrijevim hipokloritom nije dovoljno za kompletno uklanjanje *E. faecalis*, neovisno o tehnici ispiranja i količini irigansa. Iako je tijekom *PUI-ja* u korijenski kanal ubrizgana najveća količina NaOCl-a, nije postignut naj-

group three samples were without CFUs. In the PUI group and the positive control group, there were no negative cultures.

Table 3 reveals the mean, median and range of CFUs observed after the irrigation protocols and time set at 45 s. There were no significant differences between the tested irrigation techniques ($p>0.05$) and no negative cultures found in any group.

Discussion

Many manual agitation techniques and machine-assisted agitation devices have been developed and investigated in order to improve root canal irrigation (20). The present study was designed to compare the antimicrobial efficacy of the conventional syringe irrigation, the PUI and the RinsEndo system regarding the same time of irrigation (45 s) and the same amount of NaOCl used (20 ml).

The distribution of an irrigant depends proportionally on the root canal volume; in very large root canals, turbulence created during irrigation may cause arresting of the dentin debris and microorganisms (21). Therefore, in this study, single-rooted teeth with approximatively the same root canal volume were selected. *E. faecalis* was chosen as a representative endodontic pathogen because it often survives chemomechanical root canal treatment, it is associated with both primary and refractory endodontic infection (22), and is often used in similar studies (18, 23). The time taken for *E. faecalis* colonization or the biofilm formation in the root canals varies between studies (23). The longer the incubation time of the bacteria present, the more mature and clinically relevant biofilm, which explains why it is increasingly used in recent studies (18, 24). We used *E. faecalis* incubated in the root canal for 21 days (18).

It has been proven that the volume of an irrigant influences the efficacy of the root canal irrigation (25). On the other hand, the time and the concentration of an irrigant also play a significant role in the ability of an irrigant to eliminate bacteria (26). In this study, 20 ml of 3% NaOCl was the most effective when used with the RinsEndo, which also provided the highest number of negative cultures (4 of 12). Many other studies have also proven great effectiveness of RinsEndo, especially in rinsing the apical third of the canal (15, 16). According to the studies so far, acoustic streaming and cavitation of the irrigant, created during the PUI, improve cleaning efficacy (25, 27). One of the explanations of our results is the irrigation time. Low efficacy of the PUI could be due to the very short time of irrigation, which was only 20 s, compared to 3.2 min of irrigation with the RinsEndo system. Although there is no general agreement regarding the irrigation time necessary to eliminate the bacteria from the root canal, it is probably that longer exposure to NaOCl is crucial for the elimination of *E. faecalis* (28). Druttman and Stock (29) also concluded that the irrigant replacement in the root canal system during PUI is more likely to be influenced by the time than by the volume used.

The results of this study showed that 45 s of irrigation with 3% NaOCl were not sufficient for the complete eradi-

bolji antimikrobni učinak. Zbog toga se može pretpostaviti da postoji točka zasićenja te da povećanje volumena preko te granice neće poboljšati antimikrobno djelovanje u korijenskom kanalu i eliminaciju bakterija (28). Moorer i Wesselink (30) otkrili su da se klorin, koji je odgovoran za antimikrobno djelovanje NaOCl-a, potroši za dvije minute, pa je 45 sekundi vjerojatno prekratko vrijeme za antimikrobno djelovanje 3-postotnog NaOCl-a na biofilm s bakterijom *E. faecalis*. U uvjetima ovog istraživanja, sistem *RinsEndo* bio je najučinkovitiji u uklanjanju *E. faecalis* kad se koristilo 20 ml NaOCl-a. Ispiranje 3-postotnim NaOCl-om tijekom 45 sekundi nije bilo dovoljno za kompletnu eradikaciju bakterija *E. faecalis*, neovisno o tehnici ispiranja.

cation of *E. faecalis*, regardless of the irrigation technique and the amount of the irrigant used. Despite the fact that during PUI the highest amount of NaOCl was used (30 ml), it did not yield better results. Therefore, it may be suggested that there may be a point of saturation and the increase in volume beyond which bacterial elimination in the complex root canal space will no longer improve (28). In addition, Moorer and Wesselink (30) found that chlorine, which is responsible for the antimicrobial effect of NaOCl, is consumed rapidly within 2 min, so the time of 45 s is probably too short for the effective antimicrobial efficacy of 3% NaOCl against 21 days old *E. faeaclis* colonization.

Under the limitations of this *in vitro* study, the RinsEndo was the most effective irrigation technique when used with 20 ml of 3% NaOCl against *E. faecalis* colonization. The irrigation with 3% NaOCl during 45 s was not sufficient for the complete eradication of *E. faecalis*, regardless of the irrigation technique used.

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Conflicts of interest

Authors do not have conflicts of interests.

Abstract

Aim: The aim of the study was to compare the antimicrobial efficacy of three irrigation techniques after the use of standardized volume of NaOCl and with standardized time and irrigation. **Methodology:** Forty-eight single rooted teeth were inoculated with an *Enterococcus faecalis* suspension for 24 h. The remaining six canals served as negative controls. The 36 root canals were randomly distributed into three experimental groups; group 1, conventional syringe irrigation; group 2, automated-dynamic irrigation (RinsEndo); group 3, passive ultrasonic irrigation (PUI). In the first protocol, the standardized volume of 3% NaOCl (20 mL) was used and in the second protocol, and standardized irrigation time (45 seconds) was used. Samples from root canals were cultured and the colony-forming units (CFUs) were counted. **Results:** When the volume of the irrigant was standardized, RinsEndo was more effective than PUI ($p<0.01$). When the irrigation time was standardized, there were no significant differences between any irrigation techniques ($p>0.05$). The RinsEndo group had the highest percentage of minimal counts of *E. faecalis* CFUs. **Conclusions:** RinsEndo was more effective than PUI only when the volume of the irrigant was standardized. However, the RinsEndo provided higher bacterial reduction in both protocols when using the least amount of the irrigant and providing longer contact time.

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