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Antibacterial Efficacy of Rotary and Reciprocating Instrumentation Techniques

Antimikrobna učinkovitost rotacijske i recipročne tehnike instrumentacije korijenskih kanala

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Abstract

Objectives: To clinically evaluate the effectiveness of rotary (ProTaper Next [PTN]) and reciprocating (Wave One Gold [WOG]) instrumentation techniques in eliminating bacteria from the root canals of teeth with apical periodontitis during a single-visit root canal treatment. **Materials and methods:** Forty patients were randomly assigned to the WOG or PTN instrumentation techniques groups. A single-visit root canal treatment was performed on all the patients. In both groups, root canals were irrigated with 10 ml of sodium hypochlorite (NaOCl) and the final irrigation protocol was applied. Microbiological samples from the root canal were collected initially (Sample 1), after chemo-mechanical instrumentation (Sample 2), and after the final irrigation protocol (Sample 3). Quantification of bacteria at each stage of root canal treatment was performed using the culture method (colony-forming units [CFUs]), and bacterial species were identified using MALDI-TOF mass spectrometry. **Results:** There were 50 aerobic and anaerobic bacterial species identified. Chemo-mechanical root canal treatment with both instrumentation techniques and the final irrigation protocol significantly reduced the number of CFUs ($p < 0.001$). There were no statistically significant differences in antibacterial efficacy between WOG and PTN groups ($p > 0.05$). **Conclusion:** Although both root canal instrumentation techniques were highly effective in removing bacteria from the necrotic canals of teeth with chronic apical periodontitis, no complete eradication of bacteria was found in any sample.

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Introduction

The initial root canal treatment of a tooth with apical periodontitis aims to eliminate infected pulp tissue and bacterial biofilm from the complex endodontic space. Additionally, it seeks to obturate the space to initiate the healing of periapical lesion, and prevent new infections. According to previous studies, conventional chemo-mechanical root canal preparation with sodium hypochlorite (NaOCl) cannot completely remove all bacteria from the endodontic space (1–3). Significant areas of intracanal dentin, especially in the apical area or intracanal complexities, still contain infected material (microorganisms, pulp tissue, and debris), regardless of the instruments used (4–6). Therefore, in recent years,

Uvod

Cilj primarnoga endodontskog liječenja zuba s apikalnim parodontitisom jest ukloniti inficirano pulpno tkivo i bakterijski biofilm iz složenoga endodontskog prostora te zabrtviti prostor korijenskog kanala materijalom za punjenje da bi se osiguralo cijeljenje periapikalne lezije i prevenirao nastanak nove infekcije. Prema dosadašnjim istraživanjima, konvencionalna kemomehanička obrada korijenskog kanala natrijevim hipokloritom (NaOCl) ne može ukloniti sve bakterije iz endodontskog prostora (1–3). Velika područja intrakanalnog dentina, posebice u apikalnom dijelu kanala ili u intrakanalnim nepravilnostima, i dalje mogu sadržavati inficirani materijal (mikroorganizme, pulpno tkivo i dentinske ostat-

much attention has been paid to developing and investigating new irrigation protocols and advanced irrigation techniques to maximally eliminate intracanal infections and promote the healing of periapical lesions (7, 8).

Mechanical root canal instrumentation aims to shape the root canal to enhance the penetration and efficacy of a disinfection irrigant. Additionally, root canal instrumentation removes pulp tissue and reduces the bacterial count (9). There is a wide variety of Ni-Ti instrumentation systems on the market, with different designs, sizes, metallurgical characteristics, surface treatments, and consequently, different behaviors in the root canal. Continuous rotary (CR) systems are well studied and show great flexibility and elasticity, thus providing increased predictability of their performance with fewer untouched canal walls in curved root canals (10, 11). The reciprocating (R) single-file instrumentation concept provides a shorter preparation time and is therefore cost-effective. Moreover, the R instruments have undergone surface improvement procedures by electropolishing or thermal treatment, resulting in greater flexibility and resistance to cyclic fatigue (9, 12). Although the CR and R systems have been extensively researched for their shaping efficacy, there are few clinical studies on their antimicrobial efficacy. Whether single-file systems can provide sufficient quality disinfection during a shorter period of chemo-mechanical instrumentation remains controversial.

Previous studies have reported an association between a better prognosis for the healing of periapical lesions and fewer bacteria or negative cultures before obturation (13, 14). *In vitro* studies have shown no significant differences between the R and CR instrumentation systems regarding the reduction of bacteria (15, 16). According to Alves et al. (17), single-file R instruments resulted in a similar intracanal bacterial reduction as CR instruments if the irrigant volume and irrigation time were similar. Siddique and Nivedhitha (18) emphasized the necessity of randomised controlled clinical studies in this area.

This randomised clinical study aimed to evaluate the efficacy of the CR (ProTaper Next [PTN]) and the R (Wave One Gold [WOG]) instrumentation techniques in the elimination of bacteria from the necrotic canals of teeth with apical periodontitis during a single-visit root canal treatment. The null hypothesis was that there was no significant difference between the antimicrobial efficacy of PTN and WOG chemo-mechanical instrumentation.

Materials and methods

Selection of patients for the study

This randomized clinical study was approved by the Ethics Committee of the School of Dental Medicine University of Zagreb (Number of approval: 05-PA-15-2/2017) and was conducted between 2018 and 2021 at the Department of Endodontics and Restorative Dental Medicine University of

ke) bez obzira na korištenu tehniku instrumentacije (4 – 6). Zbog toga je posljednjih godina velika pozornost posvećena razvoju i istraživanju novih protokola i naprednih tehnika ispiranja kako bi se uklonila intrakanalna infekcija i osiguralno cijeljenje periapikalne lezije (7, 8).

Svrha mehaničke instrumentacije jest oblikovanje korijenskog kanala kako bi se poboljšala učinkovitost i prodor sredstva za ispiranje u dublje dijelove kanala. Uz to, instrumentacija korijenskog kanala uklanja pulpno tkivo i smanjuje broj bakterija (9). Danas je na tržištu dostupan velik broj Ni-Ti instrumenata različitog dizajna, veličina, karakteristika metala, različito tretiranih površina i posljedično različitog funkcioniranja unutar korijenskog kanala. Kontinuirani rotacijski sustavi dobro su proučeni i pokazuju veliku fleksibilnost i elastičnost, pružajući povećanu predvidljivost tijekom upotrebe s manje netaknutih intrakanalnih stijenki u zakrivljenim kanalima (10, 11). Recipročni sustavi sa samo jednim instrumentom omogućuju kraće vrijeme instrumentacije i samim time mnogo su isplativiji. Štoviše, recipročni instrumenti su tijekom same izrade podvrgnuti postupcima poboljšanja površine elektropoliranjem ili toplinskom obradom, što rezultira većom fleksibilnošću i otpornošću na ciklički zamor (9, 12). Iako su i rotacijski i recipročni sustavi opsežno istraženi zbog njihove učinkovitosti kad je riječ o oblikovanju, malo je kliničkih istraživanja o njihovoj antimikrobnoj učinkovitosti. Ostaje sporno mogu li sustavi s jednim instrumentom omogućiti dovoljno kvalitetnu dezinfekciju tijekom kraće kemomehaničke instrumentacije.

Autori dosadašnjih istraživanja otkrili su povezanost između bolje prognoze za cijeljenje periapikalne lezije i nalaza manjeg broja bakterija ili negativnih kultura u korijenskom kanalu prije punjenja (13, 14). Istraživanja *in vitro* pokazala su da nema značajne razlike između recipročnih i rotacijskih sustava kad je riječ o smanjenju broja intrakanalnih bakterija (15, 16). Prema Alvesu i suradnicima (17), recipročni sustavi s jednim instrumentom pokazali su slično smanjenje intrakanalnih bakterija kao i rotacijski instrumentacijski sustavi ako je volumen sredstva za ispiranje i vrijeme ispiranja bilo slično. Siddique i Nivedhitha (18) istaknuli su nužnost randomiziranih kontroliranih kliničkih istraživanja u tom području.

Cilj ovoga randomiziranoga kliničkoga istraživanja bio je ispitati učinkovitost rotacijske ProTaper Next (PTN) i recipročne Wave One Gold (WOG) tehnike instrumentacije u eliminaciji bakterija iz nekrotičnih korijenskih kanala zuba s apikalnim parodontitisom tijekom jednoposjetnog endodontskog liječenja. Nulla hipoteza bila je da neće biti značajne razlike u antimikrobnoj učinkovitosti između PTN i WOG tehnike instrumentacije korijenskog kanala.

Materijali i metode

Odabir pacijenata za istraživanje

Etičko povjerenstvo Stomatološkog fakulteta Sveučilišta u Zagrebu odobrilo je ovo randomizirano kliničko istraživanje (broj odobrenja: 05-PA-15-2/2017) koje se provodilo od 2018. do 2021. godine u Zavodu za endodonciju i restaurativnu dentalnu medicinu Sveučilišta u Zagrebu. Kliničko

Zagreb. This clinical study was performed according to the guidelines for randomised trials in endodontics (19).

To determine the number of patients per group, a power analysis was performed, using the Wilcoxon test with an expected reduction in the number of bacteria of 95% (with an expected standard deviation that is 50% less than the average number of bacteria), a significance level of $\alpha=0.05$ and a test power of 95 %, was necessary to include at least 15 subjects per group in the study.

The patients for this clinical study were selected from the group of patients who had been referred to an endodontist by their primary dentist. They required initial root canal treatment of a single-canal tooth with a diagnosis of necrosis. Patients were selected based on inclusion and exclusion criteria. The inclusion criteria were as follows: single-root and single-canal teeth with intact pulp chambers, intact tooth crowns (no coronary leakage), no previous root canal treatment, confirmed pulp necrosis by a sensitivity test (negative results of cold test), and clinical and radiological evidence of asymptomatic apical periodontitis. The study excluded subjects who had teeth with large carious lesions, teeth with crown or root fractures, previously endodontically treated teeth, patients who had been receiving antibiotic therapy for the last three months, symptomatic teeth, or periodontal pockets deeper than 4 mm.

Each patient signed an informed consent confirming their voluntary participation in the study. Each patient could withdraw from the study at any point during the entire study period. Finally, 40 patients (25 females and 15 males) aged between 27 and 52 years were included. In each patient, only one tooth had been endodontically treated for the purpose of this study.

Clinical procedures during single-visit root canal treatment

Single-visit root canal treatment was performed by the same dentist, a resident in endodontics (AS). After inclusion in the study, the patients were randomly divided into two groups, 'the wheel decide program' (www.wheeldecide.com), according to the instrumentation technique used:

Group 1 Reciprocating WOG instrumentation technique (Dentsply Sirona, Ballaigues, Switzerland)

Group 2 Rotary PTN instrumentation technique (Dentsply, Sirona, Ballaigues, Switzerland).

All patients participated in a single-blinded randomized study, where they were unaware of their assigned experimental group throughout the study. The clinical procedure for all patients was standardized and included 1 ampoule of Articain 4% with 1:100000 adrenaline (UbistesinTM forte, 3M ESPE, Germany). Each tooth was isolated with a rubber dam and access cavity was prepared with a sterile diamond drill (Komet Medical, Savannah, USA). The operative field was disinfected with 6% hydrogen peroxide and 2.5% NaOCl before and after access cavity preparation. A 5% sodium thiosulfate solution was used to inactivate the residual NaOCl before sampling procedure. The working length of the root canal was measured using an endometer (ES-04, E Lab) with an initial K-file instrument size of #10 or #15, depend-

istraživanje provedeno je prema smjernicama za randomizirana istraživanja u endodonciji (19)

Da bi se odredio broj pacijenata po skupini, provedena je analiza snage Wilcoxonovim testom s očekivanim smanjenjem broja bakterija od 95 % (s očekivanom standardnom devijacijom koja je 50 % manja od prosječnog broja bakterija), razina značajnosti bila je $\alpha = 0,05$ i snaga testa od 95 %, prema čemu je zaključeno da u studiju treba uključiti najmanje 15 ispitanika po skupini.

Pacijenti su za to kliničko istraživanje odabrani iz skupine onih koje je specijalistu endodoncije poslao njihov primarni doktor dentalne medicine. Svi su bili upućeni na inicijalno endodontsko liječenje jednokanalnoga zuba s dijagnozom nekroze. Kriteriji za uključivanje u studiju bili su sljedeći: jednokorijenski i jednokanalni zubi s intaktnom pulpnom komorom, intaktna zubna kruna (bez koronarnog curenja), bez prethodnog endodontskog liječenja, potvrđena nekroza pulpe testom osjetljivosti (negativni rezultati testa na hladno) te klinički i radiološki dokaz asimptomatskoga apikalnoga parodontitisa. Kriteriji za isključivanje bili su: prethodno endodontski liječeni zubi, velike karijesne lezije, zubi s prijelomima krune ili korijena, simptomatski zubi i zubi s parodontnim džepovima dubljima od 4 mm i pacijenti koji su primali antibiotsku terapiju tijekom posljednja tri mjeseca.

Svaki pacijent, koji je na temelju kriterija za uključivanje i isključivanje uključen u istraživanje, potpisao je informirani pristanak kojim je potvrdio da dobrovoljno sudjeluje, a može se povući u bilo kojem trenutku tijekom cijelog razdoblja istraživanja. Konačno, u istraživanje je uključeno 40 pacijenata (25 žena i 15 muškaraca) u dobi od 27 do 52 godine. Svakomu od njih samo je jedan zub bio endodontski liječen za potrebe ovog istraživanja.

Klinički postupci tijekom jednoposjetnoga endodontskog liječenja

Jednoposjetno endodontsko liječenje obavio je jedan doktor dentalne medicine, specijalizant endodoncije (AS). Poslije uključivanja u istraživanje pacijenti su nasumično podijeljeni u dvije skupine s pomoću softverskog programa (www.wheeldecide.com). Skupine su kreirane prema korištenoj tehnici instrumentacije:

Skupina 1.: Recipročna WOG tehnika instrumentacije (Dentsply Sirona, Ballaigues, Švicarska)

Skupina 2.: Rotacijska PTN tehnika instrumentacije (Dentsply, Sirona, Ballaigues, Švicarska).

Svi pacijenti sudjelovali su u jednostruko slijepoj randomiziranoj studiji, dakle, nisu bili obaviješteni u koju su eksperimentalnu skupinu raspoređeni. Klinički postupak za sve ispitanike bio je standardiziran i uključivao je infiltracijsku anesteziju jednom ampulom 4-postotnoga artikaina s adrenalinom 1:100.000 (UbistesinTM forte, 3M ESPE, Njemačka). Svaki zub izoliran je koferdamom, a pristupni kavitet izrađen je sterilnim dijamantnim svrdlom (Komet Medical, Savannah, SAD). Prije i poslije izrade pristupnoga kaviteta, operativno polje dezinficirano je 6-postotnim vodikovim peroksidom i 2,5-postotnim NaOCl-om. Otopina natrijeva tiosulfata (5 %) korištena je za inaktivaciju zaostalog NaOCl-a prije postupka uzimanja mikrobiološkog uzorka.

ing on the initial size of the apical foramen. Teeth with an initial size of the apical foramen greater than #15 were not included in the study. The working length was set to the apical foramen (value 0.0 on the endometer). The root canals were then instrumented using WOG (Group 1) or PTN (Group 2) chemo-mechanical preparations.

Group 1 Wave One Gold root canal instrumentation (WOG)

For the root canal instrumentation, the WOG Primary (red) 25/07 instrument (length 25 mm) was used with an endomotor (WaveOne, Maillefer Dentsply, Switzerland) set at the WAVE ONE reciprocating motion. During the instrumentation, 'in and out brushing motions' were used with continuous irrigation of the root canal with 2.5% NaOCl (total amount of 10 ml divided in four cycles). The patency of the apical foramen was assessed using a K-file size of #10 (Dentsply Sirona, USA). Indicators of adequate root canal cleaning included the presence of clean dentin debris at the tip of the instrument, clear irrigation, and firm, smooth walls.

Group 2 ProTaper Next root canal instrumentation (PTN)

For the root canal instrumentation, PTN instruments X1 (17/04) and X2 (25/06) (25 mm length) were equipped with an endomotor (WAVE ONE, Maillefer Dentsply, Switzerland) set at the following settings (rotation speed, 300 rpm; torque, 2.7 N/cm²). The instrumentation protocol was identical to that of Group 1: 2.5% NaOCl (10 ml) during instrumentation, with the same indicators of completed instrumentation. A new set of instruments was used for each patient.

After chemo-mechanical instrumentation, a final irrigation protocol was included for all patients: NaOCl (2.5 %, 5 mL) for 30 s, EDTA (15%, 2 mL) for 60 s, and NaOCl (2.5%, 5 mL) for 30 s. Root canals were irrigated with side-vented needles (Sterri Irrigation Tips, DiaDent; Netherlands) and a 2-mL syringe using the passive irrigation technique.

All root canals were filled with gutta-percha, either WOG Primary or PTN X2, along with accessory gutta-percha points. The cold lateral condensation technique was used with epoxy resin-based sealer (AH Plus, Dentsply De Tery, USA). Subsequently, the teeth were temporarily closed with glass ionomer material (Fuji IX; GC Corporation, Tokyo, Japan), and the patient was referred for a control radiograph of the tooth.

Microbiological sampling of root canals

Microbiological samples were collected from the root canals after access cavity preparation (Sample 1), after chemo-mechanical preparation (Sample 2), and after the final irrigation protocol (Sample 3). In each phase, two microbiological samples were collected using the paper-point collection technique, which involved scraping the root canal walls before-

Radna duljina korijenskog kanala izmjerena je endometrom (ES-04, E Lab, Hrvatska) s veličinom instrumenta *K-file* #10 ili #15, ovisno o početnoj veličini apikalnog foramena. Zubi s početnom veličinom apikalnog foramena većom od #15 nisu bili uključeni u istraživanje. Radna duljina mjerena je do vanjskoga apikalnog otvora (vrijednost 0,0 na endometru). Korijenski kanali zatim su kemomehanički obrađeni WOG (skupina 1.) ili PTN (skupina 2.) tehnikom instrumentacije.

Skupina 1. Wave One Gold instrumentacija korijenskih kanala (WOG)

Za instrumentaciju korijenskog kanala korišten je WOG Primary 25/07 instrument (25 mm duljine) na endomotoru (WaveOne, Maillefer Dentsply, Švicarska) postavljenom na recipročnu instrumentaciju WAVE ONE. Tijekom instrumentacije korišteni su *pokreti četkanja unutra i van* uz kontinuirano ispiranje korijenskog kanala 2,5-postotnim NaOCl-om (ukupni volumen od 10 mL podijeljen u četiri ciklusa). Prohodnost apikalnog foramena postignuta je instrumentom *K-file* veličine #10 (Dentsply Sirona, SAD). Pokazatelji zadovoljavajućeg čišćenja korijenskog kanala uključivali su prisutnost čiste, suhe dentinske piljevine na vrhu instrumenta, bistru tekućinu irigansa nakon izlaska iz korijenskog kanala i čvrste, glatke stijenke intrakanalnog dentina.

Skupina 2. ProTaper Next instrumentacija korijenskih kanala (PTN)

Za instrumentaciju korijenskog kanala korišteni su PTN instrumenti X1 (17/04) i X2 (25/06) (25 mm duljine) na endomotoru (WAVE ONE, Maillefer Dentsply, Švicarska) sa sljedećim postavkama: brzina rotacije 300 okretaja u minuti i okretni moment 2,7 N/cm². Protokol instrumentacije bio je identičan onome iz skupine 1: 2,5-postotni NaOCl (10 mL) tijekom instrumentacije, s istim kriterijima završene instrumentacije. Za svakog pacijenta korišten je novi set instrumenata.

Nakon kemomehaničke instrumentacije, završni protokol ispiranja proveden je jednako za sve pacijente: NaOCl (2,5 %, 5 mL) tijekom 30 s, EDTA (15 %, 2 mL) tijekom 60 s i NaOCl (2,5 %, 5 mL) tijekom 30 s.. Korijenski kanali ispirani su iglama s postraničnim otvorom (Sterri Irrigation Tips, DiaDent; Nizozemska) i štrcaljkom od 2 mL koristeći se tehnikom pasivne irigacije.

Svi korijenski kanali napunjeni su gutaperkom, WOG Primaryjem ili PTN X2 i akcesornim gutaperkama. Korištena je tehnika hladne lateralne kondenzacije sa sredstvom za punjenje na bazi epoksidne smole (AH Plus, Dentsply De Tery, SAD), a pristupni kaviteti privremeno su zatvoreni staklenoionomernim materijalom (Fuji IX; GC Corporation, Tokio, Japan). Pacijent je upućen za kontrolno rendgensko snimanje zuba.

Prikupljanje mikrobioloških uzoraka iz korijenskog kanala

Mikrobiološki uzorci uzeti su iz korijenskih kanala tri puta: nakon izrade pristupnog kaviteta (uzorak 1), nakon kemomehaničke obrade kanala (uzorak 2) i nakon završnog protokola ispiranja (uzorak 3). U svakoj fazi prikupljena su po dva mikrobiološka uzorka tehnikom skupljanja papirna-

hand. First, a sterile physiological solution was applied inside the cavity without overflow and distributed inside the root canal using a small sterile endodontic instrument, a Hödstrem-file (Dentsply, Sirona) size # 15. The intracanal dentinal walls were gently scraped to evenly distribute saline solution throughout the canal. Then, a sterile paper point was applied to the root canal and left in place for one min. The paper points from the root canals were then stored in thioglycolate (Merck, Darmstadt, Germany) in Eppendorf tubes. Within two hours of collection, microbiological samples were processed at the Department of Microbiology. The sterility of the operative field was checked by taking microbiological samples from the access cavity using a sterile paper point. To include teeth in the study, the mentioned control samples had to be negative according to the culture method.

Microbiological analysis using culture method and MALDI-TOF

The samples were serially diluted (1:2, then 1:10) and plated on Columbia and blood agar plates. The plates were inoculated using standard microbiological methods, after which the plates were incubated under aerobic (blood agar) and anaerobic (Columbia agar) conditions. After incubation, which lasted 48 hours for aerobes and 72–96 hours for anaerobes at a temperature of 35–37 °C, the growth of microorganisms, the number of colony forming units (CFUs) was counted and bacterial species were identified.

The bacterial species identification was additionally carried out using the matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) technique. After incubation, a very thin layer of bacteria and matrix (buffer, cyano-4-hydroxycinnamic acid concentrate) were applied to a plate with precisely defined spots. The samples were then dried at room temperature, after which a homogeneous preparation was observed. The sample was then ready for identification by inserting the plate into a MALDI-TOF mass spectrometer. The identification of each bacterial species was performed within seconds. This procedure was repeated for the bacteria that were not immediately identified.

Statistical analysis

The Wilcoxon test and chi-square test were used for the statistical analysis of the results. Statistical significance was set at $p < 0.05$. SPSS (version 29.0; IBM, Armonk, NY, USA) was used for statistical analysis.

Results

There were 50 bacterial species detected in the initial sample from all patients who participated in the study (Table 1). There were approximately 5–12 bacteria species (median 8) in each root canal initially. There was no statistically significant difference in the occurrence of bacterial species be-

tim štapićem koja je uključivala prethodno struganje stijenki korijenskog kanala. Najprije je sterilna fiziološka otopina aplicirana u kavitet bez prelijevanja i raspoređena unutar korijenskog kanala s pomoću sterilnog instrumenta Hödstrem (Dentsply Sirona, SAD) veličine # 15. Intrakanalne stijenke dentina nježno su ostrugane da bi se fiziološka otopina ravnomjerno rasporedila po cijelom korijenskom kanalu. Zatim je sterilni papirnati štapić veličine 25/06 (ProTaper Next paper point X2, Dentsply Sirona, SAD) postavljen u korijenski kanal i ostavljen na mjestu jednu minutu. Papirnati štapići iz korijenskih kanala zatim su pohranjeni u tioglikolatu (Merck, Darmstadt, Njemačka) u Eppendorfovima. Unutar dva sata od uzorkovanja, mikrobiološki uzorci obrađeni su u Zavodu za mikrobiologiju Kliničkoga bolničkoga centra Zagreb. Sterilnost operativnog polja provjerena je uzimanjem mikrobioloških uzoraka iz pristupnoga kaviteta sterilnim papirnatim štapićem (ProTaper Next paper point X2, Dentsply Sirona). U istraživanje su uključeni samo zubi kojima su kontrolni uzorci biti negativni prema metodi kulture.

Mikrobiološka analiza metodom kulture i MALDI-TOF

Uzorci su serijski razrijeđeni (1 : 2, zatim 1 : 10) i nanese ni na ploče Columbia i krvni agar. Ploče su inokulirane standardnim mikrobiološkim metodama, nakon čega su pločice inkubirane u aerobnim (krvni agar) i anaerobnim (Columbia agar) uvjetima. Nakon inkubacije, koja je trajala 48 sati za aerobe i 72 do 96 sati za anaerobe na temperaturi od 35 do 37 °C, izbrojen je rast bakterija, broj jedinica koje tvore kolonije (engl. *colony forming unit* – CFU) i identificirane su bakterijske vrste.

Identifikacija bakterijskih vrsta dodatno je provedena tehnikom spektrometrije masa s pomoću laserske desorpcije i ionizacije s matricom (engl. *Matrix Assisted LASER Desorption/Inonization Time of Flight Mass Spectrometry* – MALDI-TOF MS). Nakon inkubacije, vrlo tanak sloj bakterija i matrica (pufer, koncentrat cijano-4-hidroksicimtetne kiseline) nanese je na ploču s točno definiranim točkama. Uzorci su zatim osušeni na sobnoj temperaturi, nakon čega je promatran homogeni pripravak. Uzorak je tada bio spreman za identifikaciju umetanjem ploče u spektrometar masa MALDI-TOF. Identifikacija svake bakterijske vrste obavljena je u roku od nekoliko sekunda. Taj postupak ponovljen je za bakterije koje nisu odmah otkrivene.

Statistička analiza

Za statističku analizu rezultata korišteni su Wilcoxonov test i *hi-kvadrat* test. Statistička značajnost postavljena je 5 %. Za statističku analizu korišten je SPSS (verzija 29.0; IBM, Armonk, NY, SAD).

Rezultati

U početnom uzorku (uzorak 1) kod svih pacijenata otkriveno je 50 bakterijskih vrsta (tablica 1.). U početnom uzorku bilo je od 5 do 12 različitih bakterijskih vrsta (medijan 8) u pojedinom korijenskom kanalu. Nije bilo statistički značajne razlike u pojavi bakterijskih vrsta između PTN i WOG sku-

Table 1	Bacterial species identified in necrotic root canals of teeth with chronic apical periodontitis	<i>Streptococcus oralis</i>	<i>Porphyromonas endodontalis</i>
Tablica 1.	Bakterijske vrste otkrivene u nekrotičnim korijenskim kanalima zuba s apikalnim parodontitisom	<i>Streptococcus pneumoniae</i>	<i>Porphyromonas gingivalis</i>
		<i>Streptococcus salivarius</i>	<i>Actinomyces naeslundii</i>
		<i>Streptococcus cristatus</i>	<i>Actinomyces meyeri</i>
		<i>Streptococcus intermedius</i>	<i>Actinomyces oris</i>
		<i>Streptococcus mitis</i>	<i>Actinomyces odontolyticus</i>
		<i>Streptococcus sanguinis</i>	<i>Aggregatibacter aphrophilus</i>
		<i>Streptococcus anginosus</i>	<i>Enterococcus faecalis</i>
		<i>Streptococcus gordonii</i>	<i>Lactobacillus rhamnosus</i>
		<i>Streptococcus mutans</i>	<i>Neisseria mucosa</i>
		<i>Streptococcus constelatus</i>	<i>Parvimonas micra</i>
		<i>Staphylococcus haemolyticus</i>	<i>Rothia dentocariosa</i>
		<i>Staphylococcus hominis</i>	<i>Micrococcus luteus</i>
		<i>Streptococcus pneumoniae</i>	<i>Neisseria flavescens</i>
		<i>Staphylococcus epidermidis</i>	<i>Atopobium parvulum</i>
		<i>Prevotella dentalis</i>	<i>Bacillus muralis</i>
		<i>Prevotella intermedia</i>	<i>Bifidiobacterium dentium</i>
		<i>Prevotella denticola</i>	<i>Cutibacterium acnes</i>
		<i>Prevotella intermedia</i>	<i>Gemella morbillorum</i>
		<i>Prevotella nigrescens</i>	<i>Lactobacillus kalixensis</i>
		<i>Prevotella veroralis</i>	<i>Rhizobium radiobacter</i>
		<i>Prevotella oralis</i>	<i>Rothia aeria</i>
		<i>Prevotella buccae</i>	<i>Slackia exigua</i>
		<i>Fusobacterium naviforme</i>	<i>Veillonela parvula</i>
	<i>Fusobacterium nucleatum</i>	<i>Acinetobacter schindleri</i>	

Table 2 Number of bacterial colony forming units (CFUs) and their reduction rate after each phase of root canal treatment: after access cavity preparation, after chemo-mechanical preparation, and after final irrigation protocol

Tablica 2. Broj poraslih bakterijskih kolonija (engl. Colony forming units – CFUs) i njihovo smanjenje (%) poslije svake faze endodontskog liječenja: nakon trepanacije, nakon kemomehaničke obrade kanala i nakon završnog protokola ispiranja

Number of colonies forming units (CFUs) • Broj poraslih kolonija (engl. Colony forming units, CFUs)	Initial sample (after access cavity) • Početni uzorak (nakon trepanacije)	After chemo-mechanical preparation • Nakon kemomehaničke obrade kanala		After final irrigation protocol • Nakon završnog protokola ispiranja		P value ² • P vrijednost ²	P value ³ • P vrijednost ³
	Median (IQR) ¹	Median (IQR) ¹	Reduction rate (%) ² • Stupanj redukcije (%) ²	Median (IQR)	Reduction rate (%) ² • Stupanj redukcije (%) ²		
ProTaperNext	3000 (600 - 10000)	1000 (100 - 1000)	66.7	100 (1 - 100)	96.7	<0.001	<0.001
WaveOne Gold	3000 (50 - 14500)	1000 (20 - 1400)	66.2	105 (50 - 500)	96.5	<0.001	<0.001
P value (between groups) • P vrijednost (između skupina)	0.461	0.907		0.074			

¹ IQR: interquartile range • interkvartilni raspon

² Compared to initial sample values • U usporedbi s početnim uzorkom

³ Compared final irrigation protocol values after chemo-mechanical preparation • usporedba završnog protokola ispiranja u usporedbi s vrijednostima poslije kemomehaničke obrade kanala

tween PTN and WOG groups (chi-square test) after chemo-mechanical canal treatment ($p = 0.405$) and after the final irrigation protocol ($p = 0.364$).

There was a significant reduction in the number of CFUs after each phase of root canal treatment (after chemo-mechanical instrumentation and after final irrigation) in both groups ($p < 0.001$) (Table 2). The intergroup analysis did not show statistically significant difference between the PTN and WOG group regarding reduction of CFUs after chemo-

pine (*hi-kvadrat* test) poslije kemomehaničke obrade kanala ($p = 0,405$) i završnog protokola ispiranja ($p = 0,364$).

Zabilježeno je značajno smanjenje broja bakterijskih kolonija poslije svake faze endodontskog liječenja (nakon kemomehaničke instrumentacije i završnog protokola ispiranja) u objema skupinama ($p < 0,001$) (tablica 2.). Međugrupna analiza nije pokazala statistički značajnu razliku između PTN i WOG skupine kad je riječ o smanjenju broja kolonija nakon kemomehaničke obrade ($p = 0,907$) i nakon završnog proto-

mechanical preparation ($p=0.907$) and after final irrigation protocol ($p=0.074$) (Table 2). There was no negative result (no CFUs growth) in any patients after chemo-mechanical preparation (Sample 2) and after final irrigation protocol (Sample 3).

The intergroup analysis indicated a significantly higher percentage of anaerobic species in the initial sample in the WOG group than that in the PTN group ($p = 0.011$). In the other stages of root canal treatment (after chemo-mechanical canal preparation and after the final irrigation protocol), no significant difference in the occurrence of anaerobic or aerobic species was observed between the groups ($p=0.880$ and $p=0.124$, respectively).

Discussion

This study aimed to examine the antimicrobial efficacy of rotary (PTN) and reciprocating (WOG) instrumentation techniques in single-visit root canal treatment of necrotic single-canal teeth with apical periodontitis. The results showed that both techniques could not provide a complete elimination of bacteria in any patient. Furthermore, both techniques were similarly effective in eliminating bacteria from the root canals; therefore, the null hypothesis was accepted. Several *in vitro* and clinical studies comparing CR and R root canal instrumentation techniques have been published. In a recent clinical study by Xavier et al. (20), both the R (Wave One and Reciproc) and CR systems (ProTaper and Mtwo) demonstrated similar effectiveness in reducing endotoxins and bacteria from the root canals of teeth with primary apical periodontitis. However, neither the R nor CR techniques ensured complete removal of bacteria. The same finding was observed in the present study, where the second and third samples showed the presence of bacteria in all teeth. The initial number of bacteria in the necrotic root canal was 3×10^3 (median), which is consistent with previous studies evaluating the microbiological status of necrotic teeth with chronic apical periodontitis (21, 22). Machado et al. (21) compared the *in vitro* antimicrobial efficiency of R and CR systems and reported a reduction in the number of CFUs greater than 94%, regardless of the systems tested. In this study, chemo-mechanical root canal instrumentation provided about 66% reduction of CFUs using PTN or WOG instrumentation techniques in combination with 10 ml NaOCl. In a clinical study by Cavalli et al. (23), although no significant difference in removing bacteria and endotoxins was observed between CR (Mtwo), R (Reciproc), and hybrid techniques (Genius), the CR technique demonstrated the best reduction (95.05%), followed by the hybrid (91.85%) and R (64.68%) techniques. The benefit of reciprocating instruments was proven in retreatment of filling material over hand instruments (24, 25). The similar antimicrobial efficacy of PTN chemo-mechanical root canal instrumentation compared to that of WOG could be explained as follows: First, the volume of the used 2.5% NaOCl was standardized in both groups and was 10 ml per root canal, although the time of chemo-mechanical instrumentation was not standardized. Since the time of irrigation was not standardized in the groups, it is assumed

kola ispiranja ($p = 0,074$) (tablica 2.). Nije bilo negativnog rezultata (bez rasta bakterijskih kolonija) ni u jednom uzorku nakon kemomehaničke obrade kanala (uzorak 2) i nakon završnog protokola ispiranja (uzorak 3).

Međugrupna analiza pokazala je značajno veći postotak anaerobnih vrsta u inicijalnom uzorku (uzorak 1) u skupini WOG, nego u skupini PTN ($p = 0,011$). U ostalim fazama liječenja korijenskog kanala (nakon kemomehaničke obrade kanala i nakon završnog protokola ispiranja) nije uočena značajna razlika u pojavi anaerobnih ili aerobnih vrsta između skupina ($p = 0,880$ odnosno $p = 0,124$).

Rasprava

Cilj ovog istraživanja bio je ispitati antimikrobnu učinkovitost rotacijske (PTN) i recipročne (WOG) tehnike instrumentacije nekrotičnih jednokanalnih zuba s apikalnim parodontitisom u jednom posjetu. Rezultati su pokazali da objema tehnikama instrumentacije nije moguće osigurati potpunu eliminaciju bakterija ni kod jednog pacijenta. Nadalje, obje su tehnike instrumentacije bile podjednako učinkovite u uklanjanju bakterija iz korijenskih kanala i zato je prihvaćena nulta hipoteza. Dosad je objavljeno nekoliko *in vitro* i kliničkih istraživanja u kojima su uspoređivane CR i R tehnike instrumentacije korijenskog kanala. U nedavnom kliničkom istraživanju Xaviera i suradnika (20), recipročni (Wave One i Reciproc) i rotacijski (ProTaper i Mtwo) sustavi instrumentacije pokazali su sličnu učinkovitost u smanjenju endotoksina i bakterija iz korijenskih kanala zuba s primarnim apikalnim parodontitisom. No ni jednom tehnikom nije postignuto potpuno uklanjanje bakterija. Isti nalazi otkriveni su u ovom istraživanju, u kojemu su drugi i treći uzorak pokazali prisutnost bakterija u svim zubima. Početni broj bakterija u nekrotičnom korijenskom kanalu bio je 3×10^3 (medijan), što je u skladu s prethodnim istraživanjima u kojima su autori procjenjivali mikrobiološki status nekrotičnih zuba s kroničnim apikalnim parodontitisom (21, 22). Machado i suradnici (21) usporedili su *in vitro* antimikrobnu učinkovitost recipročnih i rotacijskih sustava i izvijestili o smanjenju broja bakterijskih kolonija za više od 94 %, bez obzira na testirane sustave instrumentacije. U ovom istraživanju je kemomehanička instrumentacija korijenskog kanala osigurala smanjenje porasta bakterijskih kolonija oko 66 % nakon primjene PTN-a ili WOG-a i korištenja instrumentacijske tehnike u kombinaciji s 10 mL NaOCl-a. U kliničkom istraživanju Cavallija i suradnika (23) iako nije uočena značajna razlika u uklanjanju bakterija i endotoksina između rotacijske (Mtwo), recipročne (Reciproc) i hibridne tehnike (Genius), rotacijska tehnika pokazala je najveće smanjenje bakterija (95,05 %), a slijede je hibridna (23) (91,85 %) i recipročna (64,68 %) tehnika. Prednost recipročnih tehnika instrumentacije dokazana je u reviziji u usporedbi s ručnim tehnikama instrumentacije (24, 25). Slična antimikrobna učinkovitost PTN kemomehaničke instrumentacije korijenskih kanala u usporedbi s WOG tehnikom u ovom istraživanju, može se objasniti na sljedeći način: volumen ko-

that the duration of NaOCl action in the root canal was not significant during the chemo-mechanical instrumentation. However, the NaOCl was refreshed couple of times in both groups. Van der Sluis et al. (26) reported that refreshment with NaOCl by intermittent irrigation improved dentin debris removal. Furthermore, in addition to concentration, the duration of contact between the irrigant and the bacteria inside the canal is also crucial for its efficacy (8, 17, 27). The more frequent introduction of a fresh volume of NaOCl after each instrument plays an important role in the eradication of intracanal infection (8). The advantages of the reciprocating technique include the possibility of mechanical treatment of the canal with only one instrument, thus leading to a shorter chemo-mechanical preparation time.

The results of this study showed that the final irrigation protocol played an important role in eliminating microorganisms from the root canal, as confirmed by other studies (8). It enhanced the final bacterial reduction rate up to 99% in both groups. In this study, the conventional technique using a syringe and needle was employed, which is acceptable for simple root canal anatomy, as confirmed by the results of other studies (8).

Bacterial species in this study were identified using the culture method and additionally using MALDI-TOF analysis, revealing 50 bacterial species in the initial samples. The most common species were *Streptococcus* spp. Rôças and Siqueira (28) found also *Streptococcus* spp to be the most abundant bacteria in root canals with apical periodontitis. In the present study, equal numbers of aerobic and anaerobic bacterial species were observed. According to previous studies, the average number of bacterial species per canal ranges from 10 to 30 (29, 30). In this study, 6–12 bacterial species were detected in each canal.

This clinical study has a few limitations. First, the sampling technique using paper points can only collect bacteria present in the main root canal and on the intracanal walls, excluding intracanal complexities such as the isthmus and lateral canals. Therefore, it may not fully represent the real clinical situation (2). Second, the quantification of bacteria in the root canal was assessed only using the culture method, which has low sensitivity and cannot detect non-cultivable bacteria. Molecular methods would provide a more precise count of bacteria; however, they may yield false-positive results. The MALDI-TOF analysis reveals bacteria species that did not grow on plates. Furthermore, the period of chemo-mechanical instrumentation could not be standardized; therefore, the conclusion of this study should be interpreted with caution because it does not provide the exact antimicrobial efficacy of PTN and WOG but rather chemo-mechanical root canal instrumentation, where NaOCl irrigation played an important role. Also, it should be noted that this study evaluated the secondary aim of root canal treatment, which is the reduction of bacteria. Further studies should focus on the primary aim of root canal treatment, which is the healing of periapical lesions. However, the results of this study have clinical significance because they showed that no chemo-mechanical root canal protocol can completely eradicate bacteria from the endodontic space of single-canal teeth.

rištenoga 2,5-postotnoga NaOCl-a bio je standardiziran u objema skupinama i iznosio je 10 mL po korijenskom kanalu, iako vrijeme kemomehaničke instrumentacije nije bilo standardizirano. Budući da vrijeme ispiranja nije standardizirano u skupinama, pretpostavlja se da vrijeme djelovanja NaOCl-a u korijenskom kanalu nije bilo značajno tijekom same kemomehaničke instrumentacije. Međutim, korijenski kanali nekoliko su puta, u ciklusima, isprani NaOCl-om u objema skupinama. Van der Sluis i suradnici (26) izvjestili su da je ispiranje kanala NaOCl-om nekoliko puta u ciklusima pospješilo uklanjanje dentinskoga debrisa. Nadalje, osim koncentracije, za učinkovitost NaOCl-a ključno je i trajanje kontakta između irigansa i bakterija unutar kanala (8, 17, 27). Češće unošenje svježih količina NaOCl-a nakon svakog instrumenta važno je u eradikaciji intrakanalne infekcije (8). Prednost recipročne tehnike jest mogućnost mehaničke obrade kanala samo jednim instrumentom, što rezultira kraćom kemomehaničkom obradom kanala.

Rezultati ovog istraživanja pokazali su da je završni protokol ispiranja bio važan u eliminaciji mikroorganizama iz korijenskog kanala, što potvrđuju i druga istraživanja (8). Nakon završnog protokola ispiranja povećan je stupanj smanjenja broja bakterija do 99 % u objema skupinama. U ovom istraživanju korištena je konvencionalna tehnika ispiranja štrcaljkom i iglom što je prihvatljiva tehnika ispiranja za jednostavnu anatomiju korijenskog kanala, a to potvrđuju i rezultati drugih istraživanja (8).

Bakterijske vrste u ovom istraživanju identificirane su metodom kulture i dodatno MALDI-TOF analizom te je otkriveno 55 bakterijskih vrsta u početnim uzorcima. Najčešće vrste bile su *Streptococcus* spp. Rôças i Siqueira (28), a također je istaknuto da je *Streptococcus* spp najzastupljenija bakterija u korijenskim kanalima s apikalnim parodontitisom. U ovom istraživanju otkriven je jednak broj aerobnih i anaerobnih bakterijskih vrsta. Prema dosadašnjim istraživanjima, prosječan broj bakterijskih vrsta po kanalu kreće se od 10 do 30 (29, 30). U ovom istraživanju otkriveno je od 6 do 12 bakterijskih vrsta u svakom kanalu.

Ovo kliničko istraživanje ima nekoliko ograničenja. Prvo, tehnikom mikrobiološkog uzorkovanja s pomoću papirnatih štapića mogu se prikupiti samo bakterije iz glavnoga korijenskog kanala i s intrakanalnih stijenki, isključujući intrakanalne složenosti kao što su istmus i lateralni kanali. Zato ne mora potpuno pokazivati stvarnu kliničku situaciju (2). Drugo, kvantifikacija bakterija u korijenskom kanalu procijenjena je samo metodom kulture koja ima nisku osjetljivost i ne može otkriti bakterije koje se ne mogu uzgajati. Molekularne metode omogućile bi preciznije brojenje bakterija, no mogu dati lažno pozitivne rezultate. MALDI-TOF analiza otkrila je vrste bakterija koje nisu rasle na pločama. Nadalje, razdoblje kemomehaničke instrumentacije nije se moglo standardizirati. Zato zaključak ovog istraživanja treba tumačiti s oprezom jer ne daje točnu antimikrobnu učinkovitost PTN-a i WOG tehnike, nego radije rezultat kemomehaničke instrumentacije gdje je ispiranje NaOCl-om imalo važnu ulogu. Također treba napomenuti da se u ovom istraživanju ispitivao sekundarni cilj endodontskog liječenja, a to je smanjenje bakterija. Buduća istraživanja trebala bi se usredotočiti

The results suggest that the choice of root canal instrumentation technique, rotary or reciprocating, does not affect the microbiological status of the root canal at the time of obturation.

Conclusion

The rotary PTN and reciprocating WOG chemo-mechanical preparation were not able to remove all bacteria from the root canal in any patient. Both techniques resulted in similar elimination of intracanal bacteria when used in combination with NaOCl.

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ti na primarni cilj liječenja korijenskih kanala, a to je cijeljenje periapikalnih lezija. Međutim, rezultati imaju i kliničko značenje jer su pokazali da ni jedan kemomehanički protokol obrade korijenskog kanala ne može potpuno eliminirati bakterije iz endodontskog prostora jednokanalnih zuba. Rezultati sugeriraju da odabir tehnike instrumentacije korijenskog kanala, rotacijske ili recipročne, ne utječe na mikrobiološki status korijenskog kanala neposredno prije punjenja.

Zaključak

Kemijsko-mehanička obrada korijenskog kanala rotacijskim PTN i recipročnim WOG instrumentacijskim sustavom nije osigurala eradikaciju bakterija iz korijenskog kanala ni kod jednog pacijenta. Obje su tehnike, u kombinaciji s NaOCl-om, pokazale sličnu antimikrobnu učinkovitost.

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Sažetak

Ciljevi: Klinički ispitati učinkovitost rotacijske [(ProTaper Next (PTN)) i recipročne [Wave One Gold (WOG)] tehnike instrumentacije u uklanjanju bakterija iz korijenskog kanala zuba s kroničnim apikalnim parodontitisom tijekom jednododnog endodontskog liječenja. **Materijali i metode:** Četrdeset pacijenata raspoređeno je metodom slučajnoga odabira u WOG ili PTN skupinu. Svi su jednododno endodontski liječeni. U objema skupinama korijenski kanali isprani su s 10 mL natrijeva hipoklorita (NaOCl) i proveden je završni protokol ispiranja. Mikrobiološki uzorci iz korijenskog kanala uzeti su nakon trepanacije zuba (uzorak 1), nakon kemijsko-mehaničke obrade (uzorak 2) i nakon završnog protokola ispiranja (uzorak 3). Metodom kulture određen je broj bakterija u svakoj fazi endodontskog liječenja, a bakterijske vrste identificirane su spektrometrijom masa MALDI-TOF. **Rezultati:** Otkriveno je 50 aerobnih i anaerobnih bakterijskih vrsta. Otkriveno je značajno smanjenje broja poraslih bakterija poslije kemomehaničke obrade korijenskog kanala objema instrumentacijskim tehnikama i poslije završnog protokola ispiranja ($p < 0,001$). Nije bilo statistički značajne razlike u antibakterijskoj učinkovitosti između WOG i PTN skupine ($p > 0,05$). **Zaključak:** Iako su obje instrumentacijske tehnike bile vrlo učinkovite u uklanjanju bakterija iz nekrotičnih korijenskih kanala zuba s kroničnim apikalnim parodontitisom, ni u jednom uzorku nije potvrđena njihova potpuna eradikacija.

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MeSH pojmovi: liječenje korijenskog kanala; periapikalni parodontitis; bakterijske infekcije; tehnike rada brzo rotirajućim uređajem

Autorske ključne riječi: rotacijska i recipročna tehnika instrumentacije, uklanjanje bakterija, nekrotični korijenski kanali, apikalni parodontitis

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