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Procjena smanjenja mikroorganizama u korijenskim kanalima nakon korištenja recipročne rotacijske instrumentacije

Evaluation of Microbial Reduction in Root Canals Instrumented with Reciprocating and Rotary Systems

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Sažetak

Svrha: Ovim istraživanjem *in vitro* željela se procijeniti učinkovitost dezinfekcije korijenskih kanala nakon instrumentacije sistemom Reciproc™ i ProTaper Universal™, uz irigaciju 1-postotnim natrijevim hipokloritom (NaOCl). **Materijali i metode:** Četrdeset kanala jednokorijenskih mandibularnih premolara inficirano je bakterijama *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* i *Candida albicans*, a dvadeset kanala ostavljeno je bez infekcije. Uzorci su nasumično podijeljeni u šest grupa (n = 10): grupa 1: ProTaper Universal™ + 1-postotni NaOCl; grupa 2 (pozitivna kontrola): ProTaper Universal™ + fiziološka otopina; grupa 3 (negativna kontrola bez mikroorganizama): ProTaper Universal™ + fiziološka otopina; grupa 4: Reciproc™ + 1-postotni NaOCl; grupa 5 (pozitivna kontrola): Reciproc™ + fiziološka otopina; grupa 6 (negativna kontrola bez mikroorganizama): Reciproc™ + fiziološka otopina. **Rezultati:** Kombinacija sistema ProTaper Universal™ i 1-postotnog natrijeva hipoklorita potpuno je eliminirala sve mikroorganizme. Rast mikroorganizama uočen je pri korištenju sistema Reciproc™ i 1-postotnog natrijeva hipoklorita. **Zaključak:** Protokoli provedeni u ovom istraživanju pokazali su da sistem Reciproc™ uz 1-postotni NaOCl nije uspio u cijelosti eliminirati iz korijenskih kanala *E. faecalis*, *P. aeruginosa*, *S. aureus* i *C. albicans*.

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Ključne riječi
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Uvod

Jedan od najvažnijih ciljeva endodontskog tretmana jest eliminirati ili znatno reducirati broj mikroorganizama u korijenskim kanalima (1). Unatoč tomu, potpuna eliminacija mikroorganizama u korijenskom kanalu težak je zadatak. Patogeni kao što su *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* i *Candida albicans* obično se nalaze u korijenskom kanalu kada endodontski zahvat ne uspije (2).

Nekoliko metoda, kao što su korištenje raznih tehnika instrumentacije kanala (3), tehnike irigacije antimikrobnim otopinama (4) i intrakanalnih medikamenata (5), opisane su kako bi se postigla učinkovita intrakanalna dezinfekcija.

Natrijev hipoklorit (NaOCl) najčešći je irigans kojim se koristimo tijekom kemomehaničke instrumentacije zbog širokog spektra antimikrobne aktivnosti te svojstva da otapa organski materijal. Učinkovitost dezinfekcije tim sredstvom povezana je s duljinom kontakta otopine i mikroorganizama te s koncentracijom otpine koja može biti od 0,5 posto do 5,25 posto (6).

Proteklih godina razvijeno je nekoliko nikal-titanijskih (NiTi) instrumenata oblikovanih za preparaciju korijenskih

Introduction

One of the most important goals of endodontic treatment is to significantly eliminate or reduce the microbial load present inside an infected root canal system (1). However, the total elimination of microorganisms in the root canal remains a difficult task. Pathogens such as *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* are frequently observed when endodontic treatment has failed (2).

Several methods, including the use of a variety of instrumentation techniques (3), irrigation schemes with antimicrobial solutions (4) and intracanal medications (5), have been described aiming at a more effective intracanal disinfection.

Sodium hypochlorite (NaOCl) is the most common irrigating solution used during chemomechanical preparation due to its broad spectrum of antimicrobial activity and its ability to dissolve organic material. The efficiency of disinfection by NaOCl is related to its contact time with the microorganisms and its concentration, which can range from 0.5% to 5.25% (6).

kanala uz neprekidnu rotaciju. Sistem ProTaper Universal™ (Dentsply Maillefer, Ballaigues, Švicarska) jedan je od popularnijih takvih endodontskih sistema na tržištu. Njegova tehnologija temelji se na postupnom korištenju iglica za čišćenje i adekvatnom oblikovanju korijenskih kanala (7,8).

Nedavno se na tržištu pojavio sistem za instrumentaciju kanala za koji je potrebna samo jedna iglica i recipročna kretnja. Sistem Reciproc™ (VDW, München, Njemačka) ima samo jednu iglicu za instrumentaciju, što uvelike smanjuje radno vrijeme i čini ga četiri puta bržim od tradicionalnoga sistema NiTi. Kraće radno vrijeme pogoduje ugodi pacijenta i terapeuta (9).

Jedini problem, i pitanje koje se nameće, jest može li jedna iglica instrumentirati i dezinficirati korijenski kanal zbog skraćenoga vremena preparacije, manje količine antimikrobne otopine i kraće izloženosti otopini (10, 11).

Iako se nekoliko dosadašnjih istraživanja fokusiralo na učinkovitost čišćenja (12), apikalno izbacivanje otpada (13) te na redukciju endotoksina (14) jednoigličnog sistema, u nekoliko studija (10, 11, 15, 16) analizirana je mogućnost smanjivanja broja mikroorganizama u kontaktu s antimikrobnom otopinom za ispiranje.

Svrha ovog istraživanja *in vitro* bila je procijeniti učinkovitost sistema Reciproc™ i ProTaper Universal™ za instrumentaciju kanala tijekom dezinfekcije korijenskih kanala inficiranih bakterijama *E. faecalis, P. aeruginoas, S. aureus* e *C. albicans* koristeći se 1-postotnim natrijevim hipokloritom.

Materijali i metode

Odabir i priprema uzorka

Nabavljeno je šezdeset intaktnih mandibularnih premolara (duljine 20 – 21 mm) s ravnim, radiološki potvrđenim jednokanalnim korijenima s formiranim apeksom. Zubi su dobiveni od Banke ljudskih zuba pri Zavodu za protetiku i oralnu maksilofacijalnu kirurgiju Stomatološkog fakulteta Federalnog sveučilišta u Pernambucu, Brazil. Nabavljanje zuba odobrilo je Etičko povjerenstvo Sveučilišnog centra za zdravstvene studije. Do uporabe zubi su bili pohranjeni u 10-postotnom formalinu (INDALABOR, Minas Gerais, Brazil).

Učinjen je trepanacijski otvor. Za određivanje radne dužine (RD) korištena je K-file iglica #10 (Dentsply-Maillefer, Ballaigues, Švicarska) koja se umetala u korijenski kanal dok nije postala vidljiva sa strane apeksa. RD je određen oduzimanjem jednog milimetra od dužine dobivene incijalnom iglicom.

Uzorci su pohranjeni u testne epruvete i svaki je posebno 30 minuta steriliziran u autoklavu na temperaturi od 121 °C. Zatim je nasumično odabrano deset uzoraka koji su uronjeni u bočice s 10 ml sterilizirane infuzije Brain Heart Infusion (BHI, Oxoid, Basingstoke, UK). Uzorci su čuvani 96 sati u inkubatoru na tremperaturi od 37°C kako bi se provjerila učinkovitost sterilizatora.

In recent years, several nickel-titanium (NiTi) instruments have been designed for root canal preparation with continuous rotation movement. ProTaper Universal™ system (Dentsply Maillefer, Ballaigues, Switzerland) is one of the most popular endodontic NiTi systems currently on the market. Its technique is based on the sequential use of files to clean and shape the root canal adequately (7,8).

Recently, a new single-file instrumentation system with reciprocating motion has been introduced. The Reciproc™ system (VDW, Munich, Germany) utilizes a single instrument to prepare the root canal, thus reducing the work time, making it four times faster than traditional NiTi systems, providing greater comfort for patient and professional (9).

A concern has been presented, however, regarding the ability of a single-file instrumentation system to disinfect the root canal, due to the shortening of preparation time of the canal, together with the lesser amount of antimicrobial agent and shorter contact time (10,11).

Thus, although several previous studies have evaluated the cleaning efficacy (12), the apical extrusion of debris (13), and the reduction of endotoxins (14) of single-file systems, only few studies (10,11,15,16) have analyzed the ability to reduce intracanal microbial agents when associated with antimicrobial irrigating solutions.

Therefore, the aim of this *in vitro* study was to evaluate the efficacy of the Reciproc™ and ProTaper Universal™ instrumentation systems to disinfect root canals infected with *E. faecalis, P. aeruginoas, S. aureus* e *C. albicans* using 1% sodium hypochlorite.

Materials and Methods

Sample selection and preparation

Sixty freshly intact human mandibular premolar teeth (length 20-21 mm), straight, with radiographically confirmed single root canal and fully formed apices, were obtained from the Human Tooth Bank of the Prosthodontics and Oral and Maxillofacial Surgery, Dental School, Federal University of Pernambuco, Brazil, for this study after approval by the Research Ethics Committee of the University's Center of Health Sciences. The teeth were stored in 10% formalin (INDALABOR, Minas Gerais, Brazil) until use.

The coronal access was performed. To determine the working length (WL), a #10 K-file (Dentsply-Maillefer, Ballaigues, Switzerland) was inserted into the root canal until it was visible at the apical foramen. The WL was calculated to be 1 mm less than the length obtained with this initial file.

The specimens were stored in glass test tubes and were individually sterilized in an autoclave at 121 °C for 30 min. Ten samples were randomly chosen and immersed totally in bottles containing 10 mL of autoclaved Brain Heart Infusion (BHI, Oxoid, Basingstoke, UK). They were kept in an incubator at 37 ± 1 °C for 96 h to check the sterilization's efficacy.

Priprema eksperimenta

Metodologiju korištenu u ovom istraživanju opisali su Câmara i suradnici (17). Za ovaj pokus upotrijebljene su prilagođene staklene posude s gumenim čepovima. Eksperimentalni sistem steriliziran je 30 minuta u autoklavu na temperaturi od 121 °C, nakon toga su posude ispunjene BHI-om (Oxoid, Basingstoke, UK) u komori za laminarni protok zraka (Veco, Piracicaba, Brazil). Sistem je 96 sati držan u inkubatoru na temperaturi od 37°C te nije uočeno zamućenje medija.

Bojenje mikroorganizama

Mikroorganizmi korišteni u ovom istraživanju nabavljeni su od tvrtke American Type Culture Collection™ (ATCC, Rockville, MD, SAD). To su bili *Enterococcus faecalis* (ATCC 6057), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 29213) i *Candida albicans* (ATCC 10231).

Kulture mikroorganizama i infekcija korijenskih kanala

Sljedeća procedura obavljena je u komori za laminarni protok zraka (Veco, Piracicaba, Brazil), a korišteni su sterilni instrumenti i materijali. Nakon toga su 24 sata izolirane kolonije čistih kultura *E. faecalis*, *P. aeruginosa*, *S. aureus* i *C. albicans* uzgojenih na 10-postotnoj ovčjoj krvi i hranidbenoj podlozi BHI (Oxoid, Basingstoke, UK), inokulirane u sterilnu 0,85-postotnu otopinu natrijeva hipoklorita. Optička gustoća bakterijske otopine prilagođena je spektrofotometrijski na otprilike 3,0 x 10^8 CFU-a (colony-forming units) mL^{-1}, a optička gustoća otopine *C. albicans* prilagođena je spektrofotometrijski na višu koncentraciju od 3,0 x 10^9 CFU-a mL^{-1}. Iz svake eksperimentalne otopine uzet je jedan ml otopine, te je pripremljena mješavina od četiri mikroorganizma.

Sterilni ekspreminetalni sistem nakon toga je otvoren. Svi korijenski kanali inficirani su, osim kanala iz negativne kontrole, s 10 µL otopine koja je sadržavala mikroorganizme. Inficirana otopina aplicirana je u trenpanacijski kanal automatskom mikropipetom (Gilson, Villiera-le-Bel, Franuska). Nakon unošenja otopine u trepanacijski kanal, korištena je K-file iglica #10 (Dentsply-Maillefer, Ballaigues, Švicarska) kako bi se inficirana otopina prenijela po cijelom RD-u. Inficirani zubi inkubirani su 48 sati na temperaturi od 37°C. Zamućenost medija tijekom inkubacije bila je pokazatelj rasta mikroorganizama. Čistoća i identifikacija bakterijskih kultura potvrđene su bojenjem po Gramu, morfologijom kolonija te rastom u Petrijevim zdjelicama: Bile Aesuclin Azide Agar (Merck KGaA, Darmstadt, Njemačka) za identifikaciju *E. faecalis*, Cetrimide Agar (Merck KGaA, Darmstadt, Njemačka) za identifikaciju *P. aeruginosa*, Mannitol Salt Phenol-red Agar (Merck KGaA, Darmstadt, Njemačka) za identifikaciju *S. aureus* i Saboraud Dextrose Agar (Merck KGaA, Darmstadt, Njemačka) za identifikaciju *C. albicans*. Ako četiri mikroorganizma nisu identificirana, eksperimentalni sistem bio je odbačen. Efikasnost ove metode inficiranja korijenskih kanala testirana je u eksperimentalnom istraživanju. Svi testovi provedeni su tri puta u aseptičkim uvjetima.

Experiment preparation

The methodology used has been described previously by Câmara et al. (17). Glass vials with rubber stoppers were adjusted for use in the present experiment. The experimental systems were sterilized in an autoclave at 121 °C for 30 min, and inside a laminar flux chamber (Veco, Piracicaba, Brazil), they were filled with BHI (Oxoid, Basingstoke, UK). Then the experimental systems were kept in an incubator at 37 ± 1 °C for 96 h and no turbidity of the medium was observed.

Microbial strains

The microorganism strains used in this experiment were obtained from the American Type Culture Collection™ (ATCC, Rockville, MD, USA): *Enterococcus faecalis* (ATCC 6057), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 29213) and *Candida albicans* (ATCC 10231).

Microbial cultures and root canal infection

The following procedures were performed inside a laminar flux chamber (Veco, Piracicaba, Brazil) using sterilized instruments and materials. Isolated 24 h colonies of pure cultures of *E. faecalis*, *P. aeruginosa*, *S. aureus* and *C. albicans* grown on 10% sheep blood plus BHI (Oxoid, Basingstoke, UK) agar plates were suspended in a sterile 0.85% NaCl solution. The suspensions of the bacteria had their optical density adjusted spectrophotometrically to approximately 3.0 x 10^8 colony-forming units (CFU) mL^{-1}, and the suspensions of *C. albicans* had their optical density adjusted spectrophotometrically (FEMTO, São Paulo, Brazil) to a higher concentration of 3.0 x 10^9 CFU mL^{-1}. From each experimental suspension, 1 mL was removed and a mixture of the four selected microorganisms was prepared.

The sterilized experimental systems were then opened. The root canals were infected, except for the negative control, with 10 µL of the suspension containing the microorganisms using an automatic micropipette (Gilson, Villiera-le-Bel, France) placed into the access cavity of each tooth. After introduction of the suspension, sterile #10 K-files (Dentsply-Maillefer, Ballaigues, Switzerland) were used to carry the microbial suspension to the WL. The infected teeth were incubated at 37 ± 1 °C for 48 h. The turbidity of the medium during the incubation period indicated microbial growth. The purity and the identification of the cultures were confirmed by Gram staining, colony morphology and the growth on Petri dishes with the media: Bile Aesuclin Azide Agar (Merck KGaA, Darmstadt, Germany) to verify the presence of *E. faecalis*, Cetrimide Agar (Merck KGaA, Darmstadt, Germany) to verify the presence of *P. aeruginosa*, Mannitol Salt Phenol-red Agar (Merck KGaA, Darmstadt, Germany) to verify the presence of *S. aureus*, and Saboraud Dextrose Agar (Merck KGaA, Darmstadt, Germany) to verify the presence of *C. albicans*. If the 4 microorganisms were not identified, the experimental system was discarded. The efficiency of the method for the infection of the root canal was observed in a pilot study. All assays were conducted in triplicate under aseptic conditions.

Eksperimentalna grupa

Inficirani zubi uklonjni su iz ekperimentalnih sistema s kontaminiranim medijem te su preneseni u staklene posude kako bi bili fiksirani na početku instrumentacije.

Uzorci su nasumično podijeljeni u dvije eksperimentalne grupe (grupe 1, 4) te četiri kontrolne (grupe 2, 3, 5, 6) s po 10 korijenskih kanala u svakoj. Instrumentirani su prema sljedećim protokolima:

Grupa 1: 1-postotni NaOCl + sistem ProTaper Universal™;
Grupa 2 (positivna kontrola): sterilna 0,85-postotna otopina NaCl-a + sistem ProTaper Universal™;
Grupa 3 (negativna kontrola, bez mikroorganizama): sterilna 0,85-postotna otopina NaCl-a + sistem ProTaper Universal™;
Grupa 4: 1-postotni NaOCl + sistem Reciproc™;
Grupa 5 (pozitivna kontrola): sterilna 0,85-postotna otopina NaCl-a + sistem Reciproc™;
Grupa 6 (negativna kontrola, bez mikroorganizama): sterilna 0,85-postotna otopina NaCl-a + sistem Reciproc™.

Ispiranje

Za ispiranje korijenskih kanala korištena je 3-mililitarska štrcaljka FCF (FCF, São Paulo, SP, Brazil) s iglom broj 30 (Injecta, Diadema, SP, Brazil).

Korišten je svježe pripremljen 1-postotni natrijev hipoklorit (Farmácia Escola Carlos Dumont de Andrade, Recife, PE, Brazil) te sterilna 0,85-postotna otopina natrijeva hipoklorita za irigaciju tijekom biomehaničke preparacije korijenskih kanala iglom broj 30 odrezanom jedan milimetar kraće od duljine do apikalnog foramena.

Biomehanička preparacija korijenskih kanala

Instrumenti ProTaper Universal™ korišteni su s pomoću električnog motora (Driller Endo-Pro Torque, Sao Paulo, Brazil) brzinom od 300 rpm u sljedećem redoslijedu: SX iglica korištena je do polovine RD-a; S1 iglica korištena je 4 milimetra kraće od apikalne dužine; iglice S1 i S2 korištene su do pune radne dužine; iglice F1 (20,07), F2 (25,08) i F3 (30,09) korištene su do punog RD-a. Korijeni su isprani s 5 ml otopine za ispiranje prije instrumentacije i nakon svake upotrebe instrumenta, tako je u ukupno trebalo 35 ml po kanalu.

Instrumenti Reciproc™ korišteni su sa suprotnim kutom na manjim brzinama (Sirona Dental Systems GmbH, Bensheim, Njemačka), a bili su spojeni na električni motor za recipročnu kinematiku (VDW Silver; VDW GmbH, München, Njemačka). Svaki korijenski kanal instrumentiran je koristeći se jednom iglicom R40 VDW Reciproc™ (VDW, München, Njemačka) broj 40 koja na vrhu ima početni taper od 0,06 na prva tri milimetra. Kanal je inicijalno ispran, a Reciprocov™ instrument korišten je polaganim pokretima od unutra prema van u amplitudi od tri milimetra laganim pritiskom prema apikalno. Nakon tri pokreta instrument je uklonjen i kanal je ispran, a instrument očišćen. Ovaj postupak ponavljao se sve dok se nije postigla puna radna dužina. Nakon završene instrumentacije kanal je još jedanput ispran. Za svaki kanal korištena je jedna iglica. Za irigaciju je bilo potrebno 10 ml otopine po kanalu.

Experimental groups

The infected teeth were removed from the experimental systems with the contaminated medium and transferred to glass vials without the medium, in order for the teeth to remain fixed at the beginning of the instrumentation.

The specimens were randomly divided into 2 experimental groups (groups 1 and 4) and 4 control groups (groups 2, 3, 5 and 6) with 10 root canals each according to the protocol used during root canal preparation, as follows:

Group 1: 1% NaOCl + ProTaper Universal™ system;
Group 2 (positive control): sterile 0.85% NaCl solution + ProTaper Universal™ system;
Group 3 (negative control, without microorganisms): sterile 0.85% NaCl solution + ProTaper Universal™ system;
Group 4: 1% NaOCl + Reciproc™ system;
Group 5 (positive control): sterile 0.85% NaCl solution + Reciproc™ system;
Group 6 (negative control, without microorganisms): sterile 0.85% NaCl solution + Reciproc™ system.

Irrigation

For irrigation of the root canal, the 3-mL FCF syringe system (FCF, São Paulo, SP, Brazil) with a 30-gauge needle (Injecta, Diadema, SP, Brazil) was used.

Freshly prepared 1% NaOCl (Farmácia Escola Carlos Dumont de Andrade, Recife, PE, Brazil), and sterile 0.85% NaCl solution were used for irrigation in biomechanical preparation of the root canals, with the 30-gauge needle demarcated at 1 mm short of the apical foramen.

Root canal biomechanical preparation

The ProTaper Universal™ instruments were operated with an electric motor (Driller Endo-Pro Torque, Sao Paulo, Brazil) at a speed of 300 rpm as follows: SX file was used to one half of the WL; S1 file was used up to 4 mm short of the apex; S1 and S2 files were used to the full WL; and F1 (20.07), F2 (25.08) and F3 (30.09) files were used to the full WL. For irrigation, 5 mL of irrigating solution was used before instrumentation and after each instrument use, and so, the total volume of irrigant was 35 mL per canal.

The Reciproc™ instruments were used with a contra angle low speed (Sirona Dental Systems GmbH, Bensheim, Germany) coupled to the electric motor for reciprocating kinematics (VDW Silver; VDW GmbH, Munich, Germany). Each root canal was instrumented with a single VDW Reciproc™ (VDW, Munique, Alemanha) R40, having a size 40 at the tip and a taper of 0.06 over the first 3mm. Initially the canal was irrigated, and then, the Reciproc™ instrument was introduced with a slow in-and-out pecking motion of about 3 mm in amplitude with a light apical pressure. After three pecking motions, the instrument was removed from the canal, cleaned and the canal was irrigated. These procedures were repeated until the WL was reached by the instrument. After instrumentation was completed, the canals were irrigated once again. Each instrument was used to prepare only one canal. The total volume of irrigating solution was 10 mL per canal.

Jedan kliničar instrumentirao je sve korijenske kanale. Nakon toga irigirani su 5-postotnom otopinom natrijeva tiosulfata (Synth®, São Paulo, Brazil) kako bi se neutralizirao NaOCl. Zabilježeno je i vrijeme instrumentacije svakog kanala.

Procjena antimikrobne aktivnosti otopine za ispiranje

a. Uzorci iz svakog korijenskog kanala testirani su kako bi se potvrdilo rastu li mikroorganizmi ili ne rastu prije i poslije konačne dezinfekcije kanala. Sterilni papirnati štapići broj 15 (Dentsply-Maillefer, Petrópolis, Brazil) uzastopno su umetani u korijenski kanal kako bi se procijenila antimikrobna aktivnost nakon dva protokola instrumentacije. Svaki papirnati štapić ostavljen je u kanalu jednu minutu: X 1 – prije biomehaničkog čišćenja i X 2 – nakon biomehaničkog čišćenja. Nakon toga uzorci su premješteni u Petrijeve zdjelice sa sljedećim hranidbenim podlogama: Bile Aesuclin Azide Agar, Cetrimide Agar, Mannitol Salt Phenol-red Agar i Saboraud Dextrose Agar.

Posude su inkubirane 48 sati na 37°C. Nakon inkubacije procijenjen je rast mikoroorganizama svjetlosnim mikroskopom (Leica Microsystems, São Paulo, Brazil) podešenim na povećanje od 400 puta.

Statistička analiza

Podatci su sažeti s pomoću apsolutne frekvencije i relativnih postotaka. Rezultati su statistički analizirani Fisherovim testom. Prihvaćena je razina značajnosti od 0,05. Korišten je program Statistical Package for the Social Sciences, verzija 21 (SPSS, Chicago, IL, SAD).

Rezultati

Učinkovitost antimikrobnih otopina

Rast mikroorganizama uočen je u svim početnim uzorcima, osim u negativnoj kontroli, pa se može zaključiti da je infekcija kanala u svim eksperimentalnim grupama bila uspješna.

U svim uzorcima s pozitivnom kontrolom, mikroorganizami su rasli prije biomehaničke obrade i poslije tog postupka, a u uzorci s negativnom kontrolom nisu.

A single operator instrumented all root canals. After the instrumentation, irrigation with 5% sodium thiosulphate solution (Synth®, São Paulo, Brazil) was used to neutralize NaOCl. The shaping time of the root canals was recorded.

Assessment of antimicrobial action of the irrigants

Samples from each root canal were tested to verify the presence or absence of the microbial growth both before and after final disinfection procedures. To assess the antimicrobial action of the instrumentation protocols, sterile paper points size 15 (Dentsply-Maillefer, Petrópolis, Brazil) were consecutively placed in the root canal. Each paper point was left in the root canal for 1 min, as follows: X1 (before biomechanical preparation) and X2 (after final disinfection). The paper points were transferred to Petri dishes containing the following media: Bile Aesuclin Azide Agar, Cetrimide Agar, Mannitol Salt Phenol-red Agar, and Saboraud Dextrose Agar.

The plates were then incubated at 37 ± 1 ºC for 48 h. After incubation, microbial growth was assessed with light microscopy (Leica Microsystems, São Paulo, Brazil) at 400×.

Statistical analysis

The categorical data were summarized by means of absolute frequency and relative percentage. The results were analyzed statistically using Fisher's exact test. A level of significance of .05 was adopted. The Statistical Package for the Social Sciences, version 21 (SPSS, Chicago, IL) was used.

Results

Irrigant antimicrobial efficacy

Microbial growth was observed in all initial samples, except for the negative control groups, demonstrating that the contamination was effective in all root canals for all experimental groups.

All positive control samples showed microbial growth before and after biomechanical preparation, whereas the negative control samples showed no microbial growth.

Tablica 1. Antimikrobna učinkovitost raznih instrumentacijskih protokola u kanalima inficiranima bakterijama *E. faecalis*, *P. aeruginosa*, *S. aureus* i *C. albicans*
Table 1 Antimicrobial efficacy of various treatment protocols in root canals infected with *E. faecalis*, *P. aeruginosa*, *S. aureus* e *C. albicans*

Group	Presence		Absence		TOTAL		P value
	n	%	n	%	n	%	
1% NaOCl + ProTaper Universal™	-	-	10	100.0	10	100.0	$p^{(1)} < 0.001$*
Saline + ProTaper Universal™ (positive control)	10	100.0	-	-	10	100.0	
Saline + ProTaper Universal™ (negative control)	-	-	10	100.0	10	100.0	
1% NaOCl + Reciproc™	10	100.0	-	-	10	100.0	
Saline + Reciproc™ (positive control)	10	100.0	-	-	10	100.0	
Saline + Reciproc™ (negative control)	-	-	10	100.0	10	100.0	
TOTAL	30	50.0	30	50.0	60	100.0	

* Statistically significant difference at .05.

U tablici 1. nalazi se antimikrobna učinkovitost u svakoj grupi, kad je riječ o *E. faecalis*, *P. aeruginosa*, *S. aureus* i *C. albicans.*

Analiza frekvencije prisutnosti ili nedostatka rasta mikroorganizama u usporedbi s odabranim tretmanom pokazala je statistički značajnu razliku ($p < 0,001$) između sistema za obradu kanala.

U grupi uzoraka koji su instrumentirani sistemom ProTaper Universal™i i isprani 1-postotnom otopinom natrijeva hipoklorita, potvrđena je potpuna eliminacija mikroorganizama. U grupi uzoraka u kojoj je korišten sistem Reciproc™, a isprani su 1-postotnim natrijevim hipokloritom, potvrđena je prisutnost svih testiranih mikoorganizama.

Slika 1. pokazuje aktivnost mikroorganizama nakon korištenja sistema ProTaper Universal™ i Reciproc™, uz irigaciju 1-postotnim natrijevim hipokloritom (A) *E. faecalis*, (B) *P. aeruginosa*, (C) *S. aureus* i (D) *C. albicans.*

Čišćenje korijenskih kanala

Srednje vrijeme korišteno za preparaciju iznosilo je 1,5 minute za sustav ProTaper Universal™ i 40 sekundi za Reciproc™. Srednja vrijednost vremena instrumentacije iznosila je 4,2 do 1,5 minute za ProTaper Universal™ i 80 sekundi za Reciproc™.

The antimicrobial efficacy of treatments in each group against *E. faecalis*, *P. aeruginosa*, *S. aureus* and *C. albicans* is shown in Table 1.

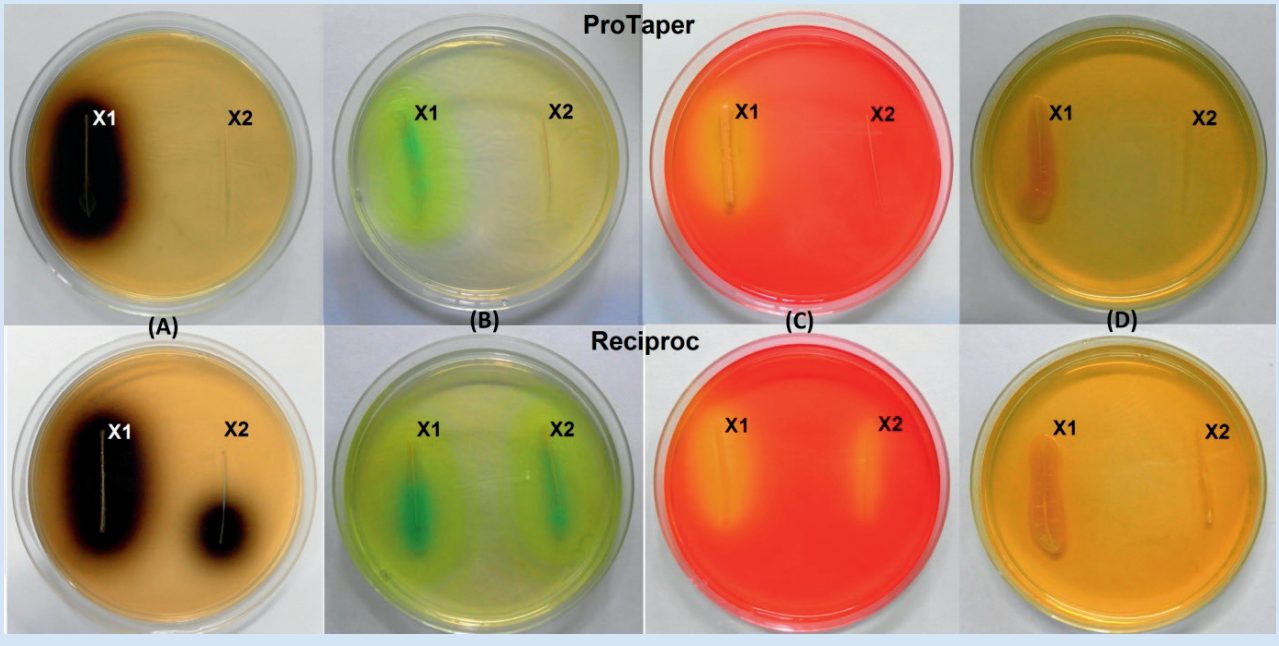
The analysis of the frequency of presence or absence of microbial growth according to the treatment applied revealed the existence of statistically significant differences ($p < 0.001$) between the instrumentation systems used.

In the group where the canals were instrumented using the ProTaper Universal™ system associated with irrigation with 1% NaOCl, the complete elimination of all microorganisms was verified. However, in the group where the Reciproc™ system associated with 1% NaOCl was used, microbial growth was observed for all microorganisms tested.

Figure 1 shows the antimicrobial activity presented by ProTaper Universal™ and Reciproc™ systems associated with 1% NaOCl against (A) *E. faecalis*, (B) *P. aeruginosa*, (C) *S. aureus*, e (D) *C. albicans*, respectively.

Root canal preparation times

The mean preparation times were 4.2 ± 1.5 minutes for ProTaper Universal™ system, and 40 ± 80 seconds for Reciproc™ system.



Slika 1. Antimikrobna aktivnost nakon upotrebe sistema ProTaper Universal™ i Reciproc™ uz irigaciju 1-postotnim NaOCl-om na mikroorganizme (A) *E. faecalis*, (B) *P. aeruginosa*, (C) *S. aureus i* (D) *C. albicans*

Figure 1 Antimicrobial activity presented by ProTaper Universal™ and Reciproc™ systems associated with 1% NaOCl against (A) *E. faecalis*, (B) *P. aeruginosa*, (C) *S. aureus*, e (D) *C. albicans*, respectively.

Rasprava

Svrha ovog istraživanja bila je procijeniti učinkovitost sistema Reciproc™ i ProTaper Universal™ uz irigaciju 1-postotnim natrijevim hipkloritom u dezinfekciji eksperimentalno inficiranih korijenskih kanala raznim patogenima.

E. faecalis, P. aeruginosa, S. aureus i *C. albicans* odabrani su kao mikrobiološki markeri zato što se smatraju najotpor-

Discussion

The purpose of this study was to evaluate the efficacy of the Reciproc™ and ProTaper Universal™ instrumentation systems together with 1% sodium hypochlorite to disinfect root canals experimentally contaminated with various pathogens.

E. faecalis, P. aeruginosa, S. aureus e *C. albicans* were selected as the microbiological markers because they are con-

nijim vrstama u inficiranim kanalima te se često povezuju s neuspjehom endodontskih terapija (2).

Od svih otopina za ispiranje najčešće se upotrebljava NaOCl zbog dokazanih svojstava u kemomehaničkoj obradi kanala. Kako se pokazalo u ovom istraživanju i u istraživanjima Gulsahija i suradnika (4), Almeide i njegovih kolega (18) i Shantiaee i njegova tima (19), NaOCl ima širok spektar antimikrobne aktivnosti, može razgraditi organski materijal te djeluje kao lubrikant tijekom instrumentacije kanala. Unatoč tomu nema zajedničkog stajališta kad je riječ o optimalnoj koncentraciji, duljini izloženosti i temperaturi natrijeva hipoklorita za kliničku primjenu u endodonciji.

Antimikrobni učinak natrijeva hipoklorita povećava se s koncentracijom otopine koja iznosi od 0,5 posto do 5,25 posto, ali uz povećanje toksičnosti (20). Koncentracija NaOCl-a korištena u ovom istraživanju bila je 1 posto. Ista koncentracija korištena je u ranijim istraživanjima (17, 21) u kojima je istaknuto da je to optimalna koncentracija za kliničku primjenu. Više koncentracije, prema riječima istih autora, nemaju bolja baktericidna svojstva te mogu negativno djelovati na periapikalna tkiva.

U nekoliko radova (4) istraživalo se optimalno vrijeme upotrebe natrijeva hipoklorita u eliminaciji mikroorganizama. Vianna i suradnici (22) istaknuli su da je potrebno 20 minuta kako bi 1-postotni NaOCl potpuno eliminirao suspenziju *E. faecalis*, *S. aureus* i *C. albicans.* Radcliffe i njegovi kolege (23) potvrdili su da je potrebno samo 10 minuta kako bi se eliminirala suspenzija *E. faecalis*. Retamozo i njegov tim (24) uočili da 1,3-postotni NaOCl ne može dezinficirati dentinske tubuluse inficirane bakterijom *E. faecalis* ni nakon 40-minutnog ispiranja. Câmara i suradnici (17) smatraju da je i nakon instrumentacije od 4,2 i 1,5 minute, 1-postotni NaOCl bio je učinkovit u dezinfekciji korijenskih kanala inficiranih bakterijama *E. faecalis, P. aeruginosa, S. aureus* e *C. albicans*. Ovi rezultati u skladu su s rezultatima našeg istraživanja u kojemu je uočena učinkovitost ove otopine u grupi uzoraka instrumentiranih sistemom ProTaper Universal™.

Tehnika instrumentacije kanala jednom iglicom predložena je kao tehnika izbora. Koncept korištenja jedne iglice u instrumentaciji kanala zanimljiv je zato što znatno smanjuje radno vrijeme pa je ugodniji i pacijentu i kliničaru. Unatoč tomu postoji problem s tim konceptom, a riječ je o mogućnosti čišćenja korijenskog kanala upravo zbog kraće instrumentacije, manje količine otopine za ispiranje i manje prodornosti otopine, što sve zajedno smanjuje mogućnost uklanjanja mikroorganizama iz korijenskih kanala (10, 11).

Rezultati ovog istraživanja pokazuju da kemomehanička instrumentacija sistemom Reciproc™ uz irigaciju 1-postotnim natrijevim hipoklooritom, nije učinkovita u eliminaciji mikoorganizama iz korijenskih kanala. Sistem ProTaper Universal™ pokazao se učinkovit u eliminaciji svih mikroorganizama iz korijenskih kanala, uz korištenje iste koncentracije irigansa.

Rezultati ovog istraživanja nisu u skladu s rezultatima istraživanja Alvesa i suradnika (10), Basmacija, Oztana i Kiyana (11), Martinha i njegovih kolega (14), Machada i njegova tima (15) te Siquere i suradnika. (16). Basmaci, Oztan i Kiyan (11) potvrdili su da nema statistički značaj-

sidered the most resistant species in infected root canals, and are often associated with endodontic treatment failures (2).

Among the irrigating solutions currently used, NaOCl is the most common and accepted worldwide due to its properties that contribute to an effective chemomechanical preparation. As demonstrated in this research as well as in studies of Gulsahi et al. (4), Almeida et al. (18) and Shantiaee et al. (19), NaOCl has a broad spectrum of antimicrobial activity, has the capacity to dissolve organic material, and acts as a lubricant during root canal system instrumentation. However, there is no general agreement regarding its optimal concentration, contact time and temperature for clinical use in Endodontics.

The antimicrobial action of NaOCl increases with its concentration, ranging from 0.5% to 5.25%, but this is also accompanied by an increase in its toxicity (20). The NaOCl concentration used in this study was 1%, since previous research (17, 21) indicates this as the optimal concentration for clinical use. Higher concentrations, according to this research, do not have better bactericidal capacity and lead to a higher degree of aggression to the periapical tissues.

Several studies (4) have evaluated the contact time required by NaOCl to eliminate different microorganisms. Vianna et al. (22) demonstrated that the time required by 1% NaOCl to eliminate suspensions of *E. faecalis*, *S. aureus*, and *C. albicans* was 20 minutes. Whereas Radcliffe et al. (23) verified that the same concentration of irrigant required 10 minutes to promote negative cultures of suspensions of *E. faecalis*. However, Retamozo et al. (24) observed that 1.3% NaOCl was not able to disinfect dentin cylinders contaminated with *E. faecalis* even after 40 minutes of irrigation. On the other hand, Câmara et al. (17) demonstrated that even after 4.2 ± 1.5 minutes of preparation time, 1% NaOCl was effective in disinfecting root canals infected with *E. faecalis, P. aeruginosa, S. aureus* e *C. albicans*. These findings corroborate the results of the present study, where the efficiency of this solution in the group in which the ProTaper Universal™ system has been used was observed.

Single-file instrumentation techniques have been proposed for the preparation of root canal systems. The concept of using a single instrument to prepare the root canal is interesting as it can considerably reduce the working time, providing greater comfort for patient and professional. However, a concern with these systems refers to their ability to disinfect the root canal, since, due to the shortening of the preparation time of the canal, minor amounts of irrigating solution, less permanence time of the solution in the canal, or all of these may hamper the eradication of all microorganisms from the root canal system (10, 11).

The results of this study demonstrated that the chemomechanical preparation using Reciproc™ system associated with 1% NaOCl was not effective in promoting samples with absence of microbial growth of the various pathogens studied. Nevertheless, the ProTaper Universal™ system was effective in eliminating all microorganisms from root canals when used the same concentration of irrigating solution.

The results of this research did not agree with the findings obtained by previous studies such as those by Alves et

ne razlike u smanjenju mikroorganizama kada se uspoređuju jednoiglična tehnika (Self-Adjusting File™ i Reciproc™) i konvencionalna tehnika s NiTi instrumentima (ProTaper™) uz irigaciju 5-postotnim NaOCl-om, 15-postotnom EDTA-om ili 7-postotnom maleičnom kiselinom. Siqueira i suradnici (16) također su potvrdili istovrsnu dezinfekciju kanala te usporedili jednoigličnu (Self-Adjusting File™ e Reciproc™) i rotacijsku tehniku (Twisted File™) u kombinaciji s 2,5-postotnim NaOCl-om kao irigansom.

Važno je istaknuti da za razliku od ostalih istraživanja, u našem smo se istraživanju za oba sistema koristili istom koncentracijom otopine za ispiranje, ali prosječno vrijeme instrumentacije kanala i količina korištene otopine za irigaciju razlikovali su se ovisno o tehnici. Za grupu uzoraka obrađenih sistemom Reciproc™ korišteno je 10 ml natrijeva hipoklorita sa srednjim vremenom istrumentacije od 40 do 80 sekundi. Za grupu uzoraka obrađenih sistemom ProTaper Universal™ korišteno je 35 ml natrijeva hipoklorita, sa srednjim vremenom instrumentacije od 4,2 do 1,5 minute.

Treba istaknuti i razliku u taperu i veličini vrha iglice između dvaju testiranih sistema instrumentacije. Oprečna su mišljenja o promjeru apikalne preparacije kanala i kako on utječe na ishod dezinfekcije kanala (14) zato što manji promjer ostavlja u kanalu previše netaknutih dijelova (25). U ovom istraživanju pokazalo se da istrument Reciproc™ R40, iako ima veći promjer (40,06), nije bolje interkanalno dezinficirao od završne iglice ProTaper Universal™ F3 s manjim promjerom (30,09), uz irigaciju 1-postotnim natrijevim hipokloritom.

Alves i suradnici (10) usporedili su jednoiglični sistem instrumentacije (Reciproc™) s mehaničko-rotacijskim sistemom NiTi (BioRaCe™) te su zaključili da su oba podjednaka zbog širine apikalne preparacije, količine korištene otopine za irigaciju te duljine same instrumentacije.

S obzirom na to možemo zaključiti da su prosječno vrijeme instrumentacije korijenskog kanala i količina otopine za ispiranje vrlo važni za smanjenje broja mikoorganizama u različitim sistemima za instrumentaciju. Ovi rezultati ističu važnost razvijanja određenih protokola koji bi se primjenjivali tijekom jednoiglične instrumentacije kako bismo ciljano postigli učinkovitiku kliničku praksu.

Zaključak

Na kraju možemo reći da su dvije testirane tehnike instrumentacije pokazale znatne razlike u dezinfekciji korijenskih kanala. Prema našem protokolu, jednoiglični sistem instrumentacije (Reciproc™) u kombinaciji s 1-postotnim NaOCl-om nije uspio potpuno eliminirati *E. faecalis, P. aeruginosa, S. aureus* i *C. albicans*.

al. (10), Basmaci, Oztan e Kiyan (11), Martinho et al. (14), Machado et al. (15) and Siquera et al. (16). Basmaci, Oztan and Kiyan (11) verified no significant differences in microbial reduction between single-file techniques (Self-Adjusting File™ e Reciproc™) and conventional Ni-Ti instrumentation (ProTaper™) when irrigation with 5% NaOCl combined with 15% EDTA or 7% maleic acid was employed. Siqueira et al. (16) also verified similar disinfecting capabilities of the single-file instrumentation systems (Self-Adjusting File™ and Reciproc™) and rotary (Twisted File™) when combined with 2.5% NaOCl irrigation.

It is important to emphasize, however, that, differently from the studies cited, in our research both instrumentation systems used the same concentration of irrigating solution, but the average root canal preparation time and the volume of irrigating solution were different, varying according to the instrumentation system employed. In the groups instrumented with the Reciproc™ system, a total of 10 mL of NaOCl with a mean instrumentation time ranging from 40 ± 80 seconds was employed. Whereas, when the ProTaper Universal™ system was used, a mean time of 4.2 ± 1.5 minutes was used to prepare the canal with 35 mL of NaOCl.

Another important point that can be emphasized is regarding the differences in taper and tip sizes existent among tested instrumentation systems. There are controversies about whether, in fact, the diameter of the apical preparation can significantly influence the outcomes of root canal disinfection (14), since smaller diameters provide a greater amount of untouched areas of the canal (25). In this study, despite the larger tip diameter presented by the Reciproc™ R40 (40.06) instrument compared to the ProTaper Universal™ F3 (30.09), allowing a slightly larger apical preparation, was not able to provide a superior intracanal disinfection when associated with 1% NaOCl.

When comparing a single-file instrumentation system (Reciproc™) with a NiTi mechanical-rotary system (BioRaCe™), Alves et al. (10) reported that the Reciproc™ system may be equivalent to other NiTi systems since the width of the apical preparation, the volume of irrigating solution and the duration of irrigation are also similar.

With respect to this, the present study found that the mean time of root canal preparation and the volume of irrigating solution used play a fundamental role in the antimicrobial effectiveness achieved during chemomechanical preparation using different instrumentation systems. Thus, the results presented here emphasize the importance of developing appropriate protocols to be obeyed during the use of single-file systems aiming a more effective clinical practice.

Conclusion

In conclusion, this study showed that the two instrumentation techniques presented significant differences in the ability to disinfect root canals. According to the protocol used, the single-file instrumentation system (Reciproc™) in combination with 1% NaOCl was not able to eliminate completely *E. faecalis, P. aeruginosa, S. aureus* e *C. albicans*.

Sukob interesa

Autori negiraju bilo kakav sukob interesa povezan s ovim istraživanjem.

Zahvale

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Conflict of interest

The authors deny any conflicts of interest related to this study.

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Abstract

Objective: This *in vitro* study aimed to evaluate the efficacy of the disinfection of root canal systems carried out with Reciproc™ and ProTaper Universal™ systems using 1% sodium hypochlorite (NaOCl). **Methods:** Forty human single-rooted mandibular premolars were infected with *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*, and twenty were not infected. The specimens were randomly divided into 6 groups (n = 10): Group 1: ProTaper Universal™ + 1% NaOCl; Group 2 (positive control): ProTaper Universal™ + saline; Group 3 (negative control without microorganisms): ProTaper Universal™ + saline; Group 4: Reciproc™ + 1% NaOCl; Group 5 (positive control): Reciproc™ + saline; Group 6 (negative control without microorganisms): Reciproc™ + saline. Samples were collected before and after the completion of specific treatments, and plated in specific media cultures. The Fisher exact test was used for the statistical analysis of differences in terms of presence or absence of microbial growth among groups. For all tested pathogens, significant differences ($p < 0.001$) were verified between the instrumentation systems used. **Results:** ProTaper Universal™ associated with 1% NaOCl completely eliminated all microorganisms. Microbial growth, however, was observed when Reciproc™ was used associated with 1% NaOCl. **Conclusion:** According to the protocol executed for this study, the Reciproc™ system associated with 1% NaOCl was not able to completely eliminate *E. faecalis*, *P. aeruginosa*, *S. aureus* and *C. albicans* from the root canal systems.

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Key words
Root Canal Irrigants; *Enterococcus faecalis*; *Pseudomonas aeruginosa*; *Staphylococcus aureus*; *Candida albicans*; sodium hypochlorite

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