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Utjecaj poliheksametilenova bigvanida na mikroorganizme u korijenskom kanalu zuba

The Effect of Polyhexamethylen Biguanide on Microorganisms in Root Canal

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Sažetak

Svrha rada: Željela se ispitati djelotvornost 0,2-postotnog poliheksametilenova bigvanida (PHMB) u eliminaciji *Enterococcus faecalis*, *Pseudomonas aeruginosa* i *Candida albicans* te ga usporediti s učinkovitošću 2,5-postotnog natrijeva hipoklorita (NaOCl). **Materijali i metode:** Na četrdeset osam jednokorijenskih zuba uklonjeni su krune i apikalni dijelovi te su korijenskih kanali instrumentirani Hedström strugačima do veličine 40. Šesnaest uzoraka inokulirano je bujonskom kulturom *E. faecalis* (ATCC 51299), *P. aeruginosa* (ATCC27853) i *C. albicans* (klinički izolat). Nakon 48-satne inkubacije i ispiranja fiziološkom otopinom uzeti su uzorci dentina iz korijenskih kanala Hedströmovim iglicama veličine 50 i to prije ispiranja 0,2-postotnim PHMB-om i 2,5-postotnim NaOCl-om i nakon toga postupka. Uzorci su zatim preneseni u epruvete s 2 mL sterilne fiziološke otopine i deseterostrukim razrjeđenjem, te su prebačeni na krvni agar nakon 30 sekundi vorteksiranja. Nakon 48-satne inkubacije na 37 ° C prebrojene su bakterijske kolonije po mililitru (CFU/ml). Za statističku analizu korišten je Kruskal-Wallisov test ($p < 0,05$) . **Rezultati:** Nakon tretmana poliheksametilenovim bigvanidom iz svih je uzoraka uklonjena *P. aeruginosa*, *E. faecalis* iz devet od jedanaest, a *C. albicans* iz sedam od jedanaest uzoraka. NaOCl je bio manje učinkovit od PHMB-a u djelovanju na *E. faecalis* ($p = 0,630$) i *P. aeruginosa* ($p = 0,138$), a posebice na *C. albicans* ($p = 0,01$). **Zaključak:** Broj *E. faecalis* i *P. aeruginosa* učinkovito smanjuju 0,2-postotni PHMB i 2,5-postotni NaOCl. Na *C. albicans* bolji antimikrobni učinak ima 0,2-postotni PHMB od 2,5-postotnog NaOCl-a. Daljnja istraživanja potrebna su kako bi se ustanovila učinkovitost PHMB-a na zreli biofilm i njegova biokompatibilnost.

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Uvod

Jedan od primarnih ciljeva endodontskog liječenja jest potpuno ukloniti i/ili smanjiti mikrobnu populaciju u korijenskim kanalima inficiranih zuba. To se postiže mehaničkom obradom, dakle uporabom strojnih ili ručnih instrumenata (1) i korištenjem irigacijskih otopina. Antimikrobne irigacijske otopine mogu doprijeti do područja nepristupačnih mehaničkom čišćenju korijenskih kanala te u dentinske tubule (2).

To je još važnije uzmemo li u obzir dubinu prodiranja u dentin nekih bakterija vrlo otpornih na kemomehaničku obradu korijenskih kanala, kao što je *E. faecalis* koji može prodrijeti u dubinu od 1.483,33 µm (hranom bogato aerobno stanje) ili 620 µm (anaerobni uvjeti bez hrane) gdje stvara mikrokolonije u obliku gljivica (3). Sve nevedeno razlog je zašto je potrebno utvrditi antibakterijsku učinkovitost materijala prije uporabe u endodontskoj terapiji (4,5).

Introduction

One of the primary objectives of root canal treatment is to reduce the microbial population in the root canals of infected teeth. This is usually accomplished by mechanical preparation using either rotary or hand files (1) along with the use of irrigant solutions. Antimicrobial irrigant solutions may reach canal ramifications and inaccessible areas and permeate completely through the dentinal tubules (2).

This is even more important if we consider the penetration depth of some very resistant and small bacteria such is *E.faecalis* which can penetrate dentinal tubules to the depth 1483.33 µm (nutrient rich aerobic condition) or 620µm (nutrition deprive anaerobic conditions). It is present as "mushroom shaped" microcolonies (3). That is the reason why is necessary to determinate the antibacterial effectiveness of materials before use in endodontic therapy (4,5).

Idealno sredstvo za irigaciju korijenskih kanala trebalo bi imati snažnu antimikrobnu aktivnost, otapati organske ostatke tkiva, dezinficirati korijenske kanale, ispirati debris iz instrumentiranih korijenskih kanala, osigurati lubrikaciju i ne bi smjelo citotoksično djelovati na periradikularno tkivo (6).

Najčešće korišteno sredstvo za irigaciju inficiranih korijenskih kanala jest natrijev hipoklorit (NaOCl) (7). Taj kemijski spoj otapa nekrotična i vitalna tkiva, ali istodobno citotoksično djeluje ako dospije do periapikalnih tkiva (8), neugodnog je mirisa i okusa, ima korozivni potencijal na instrumente (9) te može uzrokovati alergijske reakcije (10).

U ovom istraživanju korišten je Bigvasan IB10 (Arch Chemicals. Inc., Velika Britanija) s aktivnom komponentom poliheksametilenova bigvanida (PHMB) u koncentraciji od 0,2 posto. PHMB je biocid iz porodice bisbigvanida sa širokim spektrom uporabe – dezinficijens je za površine, objekte i instrumente. Također se rabi u tretmanima rana (11), za poticanje zarastanja kroničnih rana (12), u otopinama za ispiranje usne šupljine (13) te u onima za čuvanje i ispiranje kontaktnih leća (14).

Danas se zna da je u endodontskim infekcijama prisutan širok spektar mikroorganizama. Neki su gram-negativni anaerobni štapići (*Porphyromonas spp.*, *Prevotella spp.*, *Bacteroides spp.*, *Fusobacterium spp.*), zatim se pojavljuju gram-negativne bakterije u obliku spirale (*Treponema spp.*), gram-pozitivni anaerobni štapići (*Actinomyces spp.*), gram-pozitivni koki (*Streptococcus spp.*, *Enterococcus faecalis*) te gljivice, archaea, virusi (15).

Enterococcus faecalis, *Pseudomonas aeruginosa* i *Candida albicans* smatraju se vrstama najotpornijima na terapiju u inficiranim korijenskim kanalima, a često su povezani i sa sekundarnim endodontskim infekcijama (16, 17).

Svrha ovog istraživanja bila je ispitati *ex vivo* učinkovitost 0,2-postotnog PHMB-a i usporediti ga s učinkovitošću 2,5-postotnog NaOCl-a u eliminaciji rezistentnih mikroorganizama, kao što su *E. faecalis*, *P. aeruginosa* i *C. albicans*.

Materijali i metode

Istraživanje je provedeno u veljači 2011. u Zavodu za endodonciju i restaurativnu stomatologiju Stomatološkog fakulteta u Zagrebu te u Zavodu za kliničku mikrobiologiju Klinike za infektivne bolesti *Dr. Fran Mihaljević* u Zagrebu.

Prikupljeno je četrdeset i osam jednokorijenskih zuba izvađenih zbog ortodontskih ili parodontoloških razloga. Prije toga nisu bili endodontski tretirani. Nakon ekstrakcije pohranjeni su u 1-postotnom kloraminu. Kamenac i ostaci tkiva uklonjeni su kiretom i diskovima za poliranje (3M ESPE, SAD). Prije pripreme zubi su tijekom noći bili u 0,5-postotnoj otopini NaOCl-a kako bi se postigla površinska dezinfekcija. Krune i apikalni dijelovi uklonjeni su dijamantnim svrdlima s vodenim hlađenjem – krune su uklonjene na caklinsko-cementnom spojištu (CCS), a apikalni dijelovi na razini koja je odgovarala primjercima dugima 10 milimetara. Korijenski kanali instrumentirani su Hedström strugačima (SybronEndo, Orange, CA, SAD) do veličine 40, uz stalno ispiranje 2-postotnim NaOCl-om (Medika dd, Zagreb, Hr-

An endodontic irrigant should ideally exhibit powerful antimicrobial activity, dissolve organic tissue remnants, disinfect the root canal space, flush out debris from the instrumented root canals, provide lubrication and have no cytotoxic effects on the periradicular tissue (6).

The most frequently used irrigant in the treatment of the infected root canals is sodium hypochlorite (NaOCl) (7). It has solvent activity for both necrotic and vital tissues but at the same time has a cytotoxic effect when injected in the periapical tissues (8), leaves bad smell and taste, has a corrosive potential (9) and may cause allergic reactions (10).

Bigvasan IB10 (Arch Chemicals. Inc. UK) with its active component polyhexamethylen biguanide (PHMB) in concentration of 0.2% was used in this study. PHMB is biocide of bisbiguanide familiy with broad spectrum of use; disinfectant for surfaces, objects, instruments. It is also used in wound treatments (11), promoting wound healing (12), in mouthwash formulations (13) and in soft lenses care solutions (14).

Today a wide range of microorganisms is known to be a part of intraradicular infections. Some of them are Gram negative anaerobic rods (*Porphyromonas spp.*, *Prevotella spp.*, *Bacteroides spp.*, *Fusobacterium spp.*), Gram negative spiral-shaped bacteria (*Treponema spp.*), gram positive anaerobic rods (*Actinomyces spp.*), Gram positive cocci (*Streptococcus spp.*, *Enterococcus faecalis*), funghi, archaea, viruses (15).

Enterococcus faecalis, Pseudomonas aeruginosa and *Candida albicans* are considered to be the most resistant species in infected root canals and are often associated with endodontic treatment failures (16, 17).

The aim of this study was to test *ex vivo* the effectiveness of 0.2% PHMB and compare it with effectiveness of 2.5% NaOCl in the elimination of resistant microorganisms such as *E. faecalis, P. aeruginosa* and *C. albicans.*

Materials and methods

This study was performed at the Department of Endodontics and Restorative Dentistry, School of Dental Medicine, Zagreb, Croatia and at the Department of Clinical Microbiology, University Hospital for Infectious Diseases "Dr. Fran Mihaljević", Zagreb, Croatia during February 2011.

Forty- eight single rooted teeth extracted because of orthodontic or periodontal reasons were collected. Teeth were not previously endodontically treated. After extraction teeth were stored in 1% chloramine. Calculus and tissue remnants were removed with curettes and polymer discs (3M ESPE, USA). Before preparation, teeth were kept overnight in 0.5% NaOCl for surface disinfection. The crowns and apical parts were removed with diamond burrs under water cooling resulting; crowns were removed at enamel-cemento junction (ECJ) and apical parts were removed at level that corresponded with 10 mm long specimens. The root canals were enlarged with Hedström files (SybronEndo, Orange, CA, USA) to size 40 under constant irrigation with 2.5%

vatska). Zaostali sloj uklonjen je 17-postotnom EDTA-om (Pulpdent, Watertown, MA, USA) tijekom pet minuta. Cement s korijena zuba uklonjen je cilindričnim dijamantnim svrdlima pri maloj brzini (<100 okretaja u minuti), a zatim je površina korijena obložena prozirnim lakom za nokte koji se sušio pola sata na sobnoj temperaturi. Uzorci su nasumce podijeljeni u tri skupine (šesnaest zuba u svakoj). Kulture *E. faecalis* (ATCC 51299), *P. aeruginosa* (ATCC27853) i *C. albicans* (klinički izolat) pripremljene su u obliku bujona (Triptic Soja bujon, Difco). Bujonske kulture sadržavale su 5 – 9 x 10^8 kolonija mikroorganizama po mililitru (CFU / mL), što je procijenjeno serijskim razrjeđenjima svakoga bujona posebno. Uzorci za *P. aeruginosa*, *E. faecalis* i *C. albicans* uronjeni su u epruvete s po 2 ml bujonske kulture (svaki uzorak u svoju epruvetu s odgovarajućim bujonom) i inkubirani 48 sati na 37 ^{0}C. Nakon inkubacije korijenski su kanali isprani fiziološkom otopinom, a uzorci prikupljeni s unutarnjih zidova korijenskih kanala Hedström strugačima veličine 50 (uzorak *A*). Jedanaest uzoraka tretirano je 0,2-postotnim PHMB-om, a pet uzoraka 2,5-postotnim NaOCl-om. Uzorci *A* bili su negativna kontrolna skupina (isprani samo fiziološkom otopinom), a uzorci *B* koristili su se za rezultate nakon ispiranja 0,2-postotnim PHMB-om i 2,5-postotnim NaOCl-om. Tretman se sastojao od ispiranja uzoraka s 10 mL 0,2-postotnog PHMB-a (2 ml svake dvije minute) ili s 10 mL 2,5-postotnog NaOCl-a, nakon čega je slijedilo ispiranje s 2 ml neutralizatora Tween 80 (Croda zdravstvo, Yorkshire, Engleska, Velika Britanija). Uzorci dentinske piljevine preneseni su u epruvete s 2 mL sterilne fiziološke otopine i deseterostrukim razrjeđenjem. Nakon 30 sekundi vorteksiranja uzorci su nasađeni na ploče s krvnim agarom (Columbia Blood Agar, Oxoid, Velika Britanija). Nakon 48-satne inkubacije na 37 ° C prebrojene su bakterijske kolonije po mililitru (CFU/ mL) i izračunat je log10 za svaki uzorak posebno. Podaci su analizirani u SPSS verziji 16 (IBM, Novi Orchard Road, NY, SAD). Normalnost distribucije podataka ispitana je Shapiro-Wilkovim testom. Razlike između skupina uspoređivale su se Kruskall-Wallisovim testom ($p < 0,05$).

Rezultati

Uočljiva je razlika između netretirane skupine *A* i tretirane skupine *B* u uzorcima ispiranima 0,2-postotnim PHMB-om. Nakon tretmana 0,2-postotnim PHMB-om u dva uzorka s *E. faecalis* dogodilo se 2-3 log smanjenje broja mikroorganizama, a u jednomu je uočeno deseterostruko smanjenje, kako je prikazano u tablici 1. Prisutna je statistički značajna razlika između skupina *A* i *B* ($\chi2 = 14,450$, df = 1, $p = 0,0001$).

Nakon tretmana 0,2-postotnim PHMB-om u svim uzorcima s *P. aeruginosa* bakterije nisu rasle, kao što je prikazano u tablici 2. Prisutna je statistički značajna razlika između skupina *A* i *B* ($\chi2 = 18,021$, df = 1, $p = 0,0001$). Nakon tretmana 0,2-postotnim PHMB-om u sedam od jedanaest uzoraka s *C. albicans* nisu rasle gljivice, a u četiri uzorka pojavila se redukcija broja gljivica 1-2 log, kao što je prikazano u tablici 3. Vidljiva je statistički značajna razlika između skupina *A* i *B* ($\chi2 = 16,147$, df = 1, $p = 0,0001$).

NaOCl (Medika d.d, Zagreb, Croatia). Smear layer was removed by rising with 17% EDTA (Pulpdent, Watertown, MA, USA) for five minutes. The root cement was removed with cylindrical diamond bur at low speed (< 100 rpm) and root surfaces coated with transparent nail varnish which was air dried for half an hour at room temperature. Specimens were randomly divided into three groups (sixteen specimens in each group). Overnight broth cultures of *E. faecalis* (ATCC 51299), *P. aeruginosa* (ATCC27853) and *C. albicans* (clinical isolate) were prepared in tryptic soy broth (Triptic Soy Broth, Difco). The broth cultures contained 5 - 9 x 10^8 colony-forming units (CFU)/mL as estimated by subculturing serial dilutions of each broth. Specimens for *P. aeruginosa, E. faecalis* and *C. albicans* were immerged into 2 ml of broth culture (one sample in one vial with matching broth culture) . Specimens were incubated at 37 ^{0}C for 48 h. After incubation root canals were irrigated with saline, and dentine samples were collected from inner root canal walls with Hedström files size 50 (sample A). Eleven specimens were treated with 0.2% PHMB and five specimens with 2.5% NaOCl. Sample A presented negative control group (rinsed only with saline) and sample B presented results after irrigation with 0.2% PHMB, and 2.5% NaOCl. Treatment included irrigation with 10 mL of 0.2 % PHMB (2mL every two minutes) or with 10 mL of 2.5% NaOCl followed with 2 mL of neutralizator Tween 80 (Croda Health Care, Yorkshire, England, UK). Samples of dentin chips were put into vials containing 2 mL of sterile saline and tenfold dilution. After 30 s of vortexing samples were subcultured on three blood agar (Columbia Blood Agar, Oxoid, UK) plates each. After 48 h incubation at 37 °C visible colonies from appropriated dilutions were counted and average colony forming units per millilitre (CFU/mL) was calculated and log10-transformed for each sample. Data were analyzed using a SPSS version 16 (IBM, New Orchard Road, NY, USA). Normality of data distribution was tested using Shapiro-Wilk test. Groups were compared using Kruskall-Wallis test with $p<0.05$.

Results

There was a significant difference between untreated group „A“ and treated group „B“ in samples treated with 0.2% PHMB. After treatment with 0.2% PHMB, two samples that were infected with E.faecalis showed a 2-3 log reduction of microorganism and in one sample 10-fold reduction as presented in Table 1. There was statistically significant difference between group A and B (χ^2=14.450, df=1, p=0.0001).

After treatment with 0.2% PHMB in all the samples infected with *P. aeruginosa* there was no bacteria growth as shown in Table 2. There was also statistically significant difference between group „A“ and „B“(χ^2=18.021, df=1, p=0.0001). After treatment with 0.2% PHMB seven out of eleven samples that were infected with *C.albicans* remained free of growth and in four samples there was a 1- 2 log reduction of microorganisms as shown in Table 3. There was statistically significant difference between group „A“ and „B“ (χ^2=16.147, df=1, p=0.0001).

Tablica 1. Broj E. faecalis prije i poslije tretmana 0,2-postotnim PHMB-om * (CFU / mL) **
Table 1 Number of *E.faecalis* before and after treatment with 0.2% PHMB*(CFU/mL)**

N	*Enterococcus faecalis*	
	Prije tretmana • Before treatment	Nakon tretmana • After treatment
1	$1.3x10^4$	$4.5x10^2$
2	$1.5x10^4$	sterilno • sterile
3	$3.1x10^3$	sterilno • sterile
4	$1.6x10^4$	sterilno • sterile
5	$2.4x10^3$	sterilno • sterile
6	$2.5x10^3$	sterilno • sterile
7	$2.2x10^4$	sterilno • sterile
8	$1.5x10^2$	sterilno • sterile
9	$1.1x10^2$	sterilno • sterile
10	$4.9x10^4$	$1.5x10^1$
11	$2.2x10^3$	$1.3x10^3$

Tablica 2. Broj P. aeruginosa prije i poslije tretmana 0,2-postotnim PHMB-om *
Table 2 Number of *P.aeruginosa* before and after treatment with 0.2% PHMB*

N	*Pseudomonas aeruginosa*	
	Prije tretmana • Before treatment	Nakon tretmana • After treatment
1	$2.2x10^4$	sterilno • sterile
2	$1.1x10^2$	sterilno • sterile
3	$3.1x10^3$	sterilno • sterile
4	$2.4x\ 10^4$	sterilno • sterile
5	$1.3x10^3$	sterilno • sterile
6	$3.1x10^2$	sterilno • sterile
7	$4.8x10^3$	sterilno • sterile
8	$2.5x10^3$	sterilno • sterile
9	$1.6x10^2$	sterilno • sterile
10	$2.3x10^3$	sterilno • sterile
11	$2.5x10^4$	sterilno • sterile

Tablica 3. Broj C. albicans prije i poslije tretmana 0,2-postotnim PHMB-om * (CFU / mL) **
Table 3 Number of *C.albicans* before and after treatment with 0.2% PHMB* (CFU/mL)**

N	*Candida albicans*	
	Prije tretmana • Before treatment	Nakon tretmana • After treatment
1	$1x10^3$	$7.5x10^1$
2	$4x10^3$	$1x10^1$
3	$1.5x10^3$	sterilno • sterile
4	$1x10^2$	$2x10^1$
5	$7x10^2$	sterilno • sterile
6	$2x10^3$	sterilno • sterile
7	$3x10^3$	sterilno • sterile
8	$3x10^2$	$1x10^1$
9	$2x10^2$	sterilno • sterile
10	$1x10^3$	sterilno • sterile
11	$2.2x10^3$	$1x10^1$

Tablica 4. Broj E. faecalis prije i poslije tretmana 2,5-postotnim NaOCl-om *** (CFU / mL) **
Table 4 Number of *E.faecalis* before and after treatment with 2.5% NaOCl*** (CFU/mL)**

N	*Enterococcus faecalis*	
	Prije tretmana • Before treatment	Nakon tretmana • After treatment
1	$3,5x10^4$	$1,2x10^3$
2	$1,6x10^4$	sterilno • sterile
3	$1,3x10^3$	sterilno • sterile
4	$1,0x10^4$	$2,5x10^2$
5	$4,0x10^4$	sterilno • sterile

Tablica 5. Broj P. aeruginosa prije i poslije tretmana 2,5-postotnim NaOCl-om *** (CFU / mL) **
Table 5 Number of *P.aeruginosa* before and after treatment with 2.5% NaOCl*** (CFU/mL)**

N	*Pseudomonas aeruginosa*	
	Prije tretmana • Before treatment	Nakon tretmana • After treatment
1	$1,8x10^2$	sterilno • sterile
2	$3,1x10^3$	sterilno • sterile
3	$2,2x10^3$	sterilno • sterile
4	$2,5x10^3$	$1,2x10^2$
5	$1,9x10^4$	sterilno • sterile

Tablica 6. Broj C. albicans prije i poslije tretmana 2,5-postotnim NaOCl-om*** (CFU / mL) **
Table 6 Number of *C.albicans* before and after treatment with 2.5% NaOCl*** (CFU/mL)**

N	*Candida albicans*	
	Prije tretmana • Before treatment	Nakon tretmana • After treatment
1	$4x10^3$	$3x10^2$
2	$1,8x10^2$	$1,2x10^1$
3	$3,2x10^3$	$1,5x10^1$
4	$1,5x10^3$	$1x10^2$
5	$3x10^2$	$2x10^1$

* PHMB – Poliheksametilenov bigvanid • Polyhexamethylen biguanide
** CFU/mL – Broj kolonija po mililitru • Colony forming units per milliliter
*** NaOCl – Natrijev hipoklorit • Sodium hypochlorite

Kod uzoraka ispiranih 2,5-postotnim NaOCl-om dobiveni su slični rezultati. Uočava se statistički značajna razlika između netretirane skupine *A* i tretirane skupine *B*. Rezultati za uzorke s *E. faecalis* pokazali su 1-2 log smanjenje mikroorganizama u dva uzorka, a u tri od njih nije bilo rasta mikroorganizama (χ^2 = 6,988, df = 1, p = 0,008) (tablica 4.). Nakon tretmana 2,5-postotnim NaOCl-om uzorci s *P. aeruginosa* također su pokazali smanjenje broja mikroorganizama u jednom uzorku, te potpuni izostanak rasta u četiri ostala (χ^2 = 7.258, df = 1, p = 0,007) (tablica 5). Uzorci s *C. albicans* pokazali su smanjenje broja mikroorganizama u svih pet uzoraka (χ^2 = 5.312, df = 1, p = 0,021) (tablica 6.).

U usporedbi učinkovitosti 0,2-postotnog PHMB-a i 2,5-postotnog NaOCl-a na *E. faecalis* i *P. aeruginosa* nije bilo statistički značajne razlike (χ^2 = 0,232, df = 1, P = 0,630, χ^2 = 2,2 df = 1, p = 0,138). S druge strane 0,2-postotni PHMB imao je statistički znatno bolje rezultate u djelovanju na *C. albicans* od 2,5-postotnog NaOCl-a (χ^2 = 6,6, df = 1, p = 0,01).

Rasprava

Uklanjanje mikroorganizama i nekrotičnog tkiva iz korijenskih kanala nužno je za uspješan ishod endodontskog liječenja. Nakon mehaničkog uklanjanja pulpe velik broj mikroorganizama i dalje ostaje u korijenskim kanalima. Zadaća sredstava za irigaciju korijenskih kanala jest njihova dezinfekcija. Iako je NaOCl trenutačno *zlatni standard* u kemijskoj obradi korijenskih kanala i dalje se trebaju pronalaziti nova sredstva. NaOCl ima širok spektar antimikrobne aktivnosti i razgrađuje organsko tkivo. Ne postoji opći dogovor o optimalnoj koncentraciji NaOCl-a – može biti između 0,5 posto i 5,25 posto (18). Veće koncentracije su učinkovitije, ali su i toksičnije pa mogu iritirati periapikalna tkiva (19).

Poliheksametilenov bigvanid (PHMB) brzo je djelujući biocid širokoga spektra djelovanja koji u niskoj koncentraciji učinkovito uništava gram-pozitivne i gram-negativne bakterije te je virucid širokog spektra. PHMB je stabilan u širokom rasponu pH (1-11), slabo se pjeni i ne sadržava formaldehid. Također je važno da je to tekućina bez mirisa i boje. Uobičajene koncentracije koje se primjenjuju prema preporukama proizvođača iznose 0,2 posto, 0,4 posto, 0,8 posto i 1 posto. Primarni su ciljevi antibakterijskog djelovanja PHMB-a vanjska i citoplazmatska membrana stanice. PHMB adherira na membrane ciljne stanice i oštećuje ih, pa iz njih izlaze ioni kalija i druge komponente stanične citoplazme, što rezultira staničnom smrću (20). 0,2-postotni PHMB već je u uporabi kao sastavni dio otopina za usnu šupljinu jer se pokazalo da je dovoljno učinkovit u sprječavanju plaka, a istodobno ne oštećuje sluznicu (13). Proizvođač te otopine predlaže i uporabu 0,2-postotne koncentracije za površinske tretmane teških infekcija.

Četrdeset osam sati inkubacije, prema Portenieru i suradnicima (21) te Foleyu i njegovim kolegama (22), dovoljno je da bi bakterije stvorile nezreli biofilm u korijenskom kanalu. Svaki od mikroorganizama korištenih u ovom istraživanju stvarao je zasebni biofilm.

E. faecalis predstavnik je gram-pozitivnih bakterija i najčešće je izolirana vrsta iz korijenskih kanala povezana s neu-

For samples treated with 2.5% NaOCl were obtained similar results. There was statistically significant difference between untreated group "A" and treated group "B". Results for samples infected with *E.faecalis* showed 1-2 log reduction of microorganisms in two samples and three of them remained free of growth (χ^2=6,988, df=1, p=0.008) (Table 4). After treatment with 2.5% NaOCl samples infected with *P.aeruginosa* also showed reduction in one sample and there was no bacteria growth in four samples (χ^2=7,258, df=1, p=0.007) (Table 5). Samples infected with *C. albicans* showed reduction of microorganisms in all five specimens (χ^2=5,312, df=1, p=0.021) (Table 6).

Compared the efficacy of 0.2% PHMB and 2.5% NaOCl on *E.faecalis* and *P.aeruginosa* there was no statistically significant difference (χ^2=0,232, df=1, P=0.630, χ^2=2,2, df=1, p=0.138). On the other side 0.2% PHMB showed statistically significant better results in reduction of *C.albicans* after treatment than 2.5% NaOCl did (χ^2=6,6, df=1, p=0.01).

Discussion

Elimination of microorganisms and necrotic tissue from the root canal system is essential for successful outcome of endodontic treatment. After mechanical removing of the pulp tissue, a large number of microorganisms still persist in the root-canal system. The role of endodontic irrigant solutions is to disinfect the root canal system. Although NaOCl solution is a „gold standard" in chemical preparation of root canal there is still need for finding new alternatives. NaOCl has a broad spectrum of antimicrobial activities and has the ability to dissolve organic tissue. There is no general agreement regarding its optimal concentration which ranges from 0.5% to 5.25% (18). Higher concentrations are more effective but have an increased toxicity and can irritate periapical and periodontal tissues (19).

Polyhexamethylen biguanide (PHMB) is a fast acting, broad spectrum biocide effective at low concentration against Gram positive and Gram negative bacteria, and is a broad spectrum virucide. PHMB is stable over a wide pH range (1-11), has low foaming properties and is formaldehyde free. What is also important it is an odour free and transparent solution. Usual concentrations that are in use by the recommendation of manufacturer are 0.2 %, 0.4%, 0.8% and 1%. The primary targets for PHMB' s antibacterial action appear to be the outer and cytoplasmic membranes. PHMB is thought to adhere to and disrupt target cell membranes, causing them to leak potassium ions and other cytosolic components which results in cell death (20). 0.2% PHMB is already in use as a mouthwash formulation and it was shown to be effective enough against plaque formation and at the same time not harmful for oral mucosa (13). Manufacturer of this solution also suggests the use of a 0.2% concentration as topical treatment in severe infections.

Forty-eight hour incubation is a time long enough for bacteria to create an immature biofilm in root canal according to Portenier et al. (21) and Foley et al. (22). Microorganisms in this study are forming three monospecies biofilms.

spjelim liječenjem (23). Uglavnom se naseljava u dentinskim tubulusima, istmusu i drugim dijelovima endodontskog sustava (24). Kako ističu Bystro i suradnici (25), može preživjeti u teškim uvjetima, poput slanih koncentracija i visokog pH. Portenier i njegovi kolege zaključili su da *E. faecalis* može preživjeti i u teškim prehrambenim uvjetima (26). U ovom istraživanju PHMB je pokazao sposobnost uklanjanja *E. faecalis* iz gotovo svih inficiranih korijenskih kanala i smanjenje njegova broja na minimum. Uzorci tretirani 2,5-postotnim NaOCl-om pokazali su slične rezultate.

P. aeruginosa predstavnik je gram-negativnih bakterija i najotporniji je član gram-negativne porodice. Uzrokuje otporne endodontske infekcije (27) i njegova morfologija vrlo je slična drugim gram-negativnim štapićima koji se obično nalaze u endodontskim infekcijama (28). Estrela i suradnici (29) izvijestili su da nakon 72-satnog kontakta $Ca(OH)_2$ pokazuje antimikrobni učinak na *P. aeruginosa*. Pallotta i njegovi kolege (30) otkrili su da $Ca(OH)_2$ i jodidov kalij-jodid (IKI) nisu mogli ukloniti *P. aeruginosa,* kao ni 0,5-postotni NaOCl te 2-postotni klorheksidin, prema Ashrafu i suradnicima (31). U našem je istraživanju 0,2-postotni PHMB eliminirao *P.aeruginosa* iz svih uzoraka. S druge strane 2,5-postotni NaOCl uspješno je uklonio *P. aeruginosa* iz četiri od pet uzoraka i pokazao dobar antimikrobni učinak na odabranu bakteriju, ali nije dovoljno dobar kao 0,2-postotni PHMB.

C. albicans je najreprezentativnija gljivica izolirana iz korijenskog kanala i ima najveću ulogu u neuspješnim endodontskim liječenjima. Također je jedan od najupornijih mikroorganizama u korijenskom kanalu i vrlo ga je teško potpuno ukloniti. Bodrumlu i suradnici (32) izvijestili su o premaloj učinkovitosti tetraciklina i NaOCl-a na *C. albicans*. U ovoj studiji je 0,2-postotni PHMB pokazao odlične rezultate u eliminaciji *C. albicans* iz inficiranih uzoraka. U većini uzoraka gljivice su bile potpuno iskorijenjene, a u ostalima je broj značajno smanjen. Istodobno 2,5-postotni NaOCl uspio je smanjiti broj *C. albicans* u svih pet uzoraka, ali nije ih bilo moguće potpuno iskorijeniti ni iz jednoga uzorka.

Na temelju rezultata ovog istraživanja može se zaključiti da 0,2-postotni PHMB dobro antimikrobno djeluje na odabrane mikroorganizme (*E. faecalis*, *P. aeruginosa* i *C. albicans)*. U usporedbi s 2,5-postotnim NaOCl-om, bolje rezultate pokazao je 0,2-postotni PHMB, osobito na uzorcima s *C. albicans*. Potrebna su daljnja istraživanja o učinkovitosti PHMB-a na zreli biofilm i o njegovoj biokompatibilnosti.

Sukob interesa

Autori negiraju sukob interesa.

E.faecalis is a representative for Gram positive bacteria and is the most frequently isolated species from teeth associated with failed root canal treatment (23). It has ability to invade dentinal tubules, isthmuses, and other ramifications of a root canal system (24). Also has the ability to survive harsh environments such as salt concentrations and are able to survive prolonged periods in high alkalinity according to Bystrom et al. (25) Portenier et al. reported that *E.faecalis* survived in harsh nutrient conditions (26). In this study PHMB showed ability to eradicate *E.faecalis* from almost all of the infected root canals and to decrease their number to minimum number. Specimens treated with 2.5% NaOCl showed similar results.

P.aeruginosa is a representative for Gram negative bacteria and it is the most resistant member of Gram negative family. It is causing persistent endodontic infections (27) and its morphology is highly similar to other Gram negative rods commonly found in endodontic infections (28). Estrela et al. (29) reported that $Ca(OH)_2$ showed antimicrobial effects on *P.aeruginosa* after 72 hours contact. Pallotta et al. (30) found that $CaOH_2$ and iodide potassium iodine were not able to eliminate *P.aeruginosa* as same as 0.5% NaOCl, and 2% chlorhexidine according to Ashraf et al. (31). In our study 0.2% PHMB eradicated *P.aeruginosa* from all specimens. On the other side 2.5% NaOCl successfully killed *P.aeruginosa* in four out of five specimens and showed good antimicrobial effects on selected bacteria but yet not good as 0.2% PHMB.

C.albicans is the most representative fungus isolated from root canal system and has the greatest role in endodontic treatment failure. It is also one of the most persistant microorganisms in root canal and is very hard to remove it completely. Bodrumlu et al. (32) reported lack of effectiveness of tetracycline and NaOCl on *C.albicans*. In this study 0.2% PHMB showed excellent results in elimination of *C.albicans* from infected specimens. In most of the specimens it was completely eradicated and in the rest of specimens it was significantly reduced. In contrary 2.5% NaOCl manage to reduce number of *C.albicans* in all of five specimens but it was not able to completely eradicate it from either of one specimen.

In conclusion based on the results of this study, it may be concluded that 0.2% PHMB has good antimicrobial effects on selected microorganisms (*E.faecalis*, *P.aeruginosa* and *C.albicans*. Compared with 2.5% NaOCl, 0.2% PHMB showed better results especially on samples infected with *C.albicans*. Further studies are necessary to determine PHMB's effectiveness on mature biofilm in root canal and it's cytotoxicity as well.

Conflict of interests

All authors deny any conflicts of interest.

Abstract

Objective of work: The objective of this study was to test the effectiveness of 0.2% polyhexamethylen biguanide in the elimination of *Enterococcus faecalis, Pseudomonas aeruginosa* and *Candida albicans* and compare it with effectiveness of 2.5% sodium hypochlorite. **Materials and Methods:** Crown and apical portion of forty- eight single-rooted human teeth was removed and the root canals instrumented with Hedström files up to size 40. Sixteen specimens were inoculated with an overnight broth culture of *E.faecalis* (ATCC 51299), *P.aeruginosa* (ATCC27853) and *C.albicans* (clinical isolate). After 48 h incubation and irrigation with saline, dentine samples were collected from root canal walls with Hedström files size 50 before and after irrigation with 0.2% PHMB and 2.5% NaOCl followed by irrigation with neutralizer and saline. Samples were put into vials containing 2 mL of sterile saline and tenfold dilution, and plated on blood agar after 30 s vortexing. Colony forming units (CFU) were counted after 48 h incubation at 37ºC. For statistical analysis Kruskal-Wallis test ($p<0.05$) was used. **Results:** After treatment with PHMB *P.aeruginosa* was eradicated from all samples, *E.faecalis* was also reduced and *C.albicans* was not grown in seven out of eleven samples. NaOCl was less effective than PHMB on *E.faecalis* ($p=0.630$) and *P.aeruginosa* ($p=0.138$), especially in case of *C.albicans* ($p=0.01$). **Conclusions:** 0.2% PHMB effectively reduced the number of *E.faecalis* and *P.aeruginosa* as well as 2.5% NaOCl). On *C.albicans* 0.2% PHMB showed better antimicrobial effect than 2.5% NaOCl. Further studies will be necessary to determine PHMB's effectiveness on mature biofilm and its biocompatibility.

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Dental Pulp Cavity; Enterococcus faecalis; Pseudomonas aeruginosa; Candida albicans; Root Canal Irrigants; Polyhexamethilen biguanide; Sodium Hypochlorite

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