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Short-Term Antibacterial Efficacy of Three Bioceramic Root Canal Sealers Against *Enterococcus Faecalis* Biofilms

Antibakterijska učinkovitost triju biokeramičkih materijala za punjenje korijenskih kanala na biofilm s bakterijom Enteroccocus faecalis

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Abstract

Objectives: The aim of the study was to evaluate the antimicrobial efficacy of three bioceramic root canal sealers against *Enterococcus faecalis (E. faecalis)* biofilm. **Material and methods:** *E. faecalis* bacterial suspension was grown on filter paper discs on agar plates. After the incubation period, the discs were covered with four different root canal sealers: 1) Premixing bioceramic root canal sealer (TotalFill BC Sealer); 2) Dual component bioceramic sealer (BioRoot RCS); 3) Mineral trioxide agreggate based sealer (MTA Fillapex); 4) Epoxy resin-based selar (AH Plus). After contact time of 60 minutes, the sealers were removed, and the discs were transferred into sterile tubes containing phosphate buffered saline. After serial dilutions, the aliquots of the suspension were cultivated for 24 hours. After the incubation period, the colony forming units (CFUs) were counted. **Results:** There were no significant differences in antibacterial efficacy between the Total Fill BC Sealer and the AH Plus sealer (p=0.386). Both sealers showed better antibacterial efficacy compared to the BioRoot RCS and the MTA Fillapex (p<0.001). **Conclusion:** The Total Fill BC Sealer and AH Plus had better antibacterial efficacy than the BioRoot RCS and the MTA Fillapex sealers.

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Key words
Anti-Infective Agents; *Enterococcus faecalis*; Root Canal Filling Materials; Organically Modified Ceramics

Introduction

The main goal of root canal treatment is the elimination of intracanal infection and obturation of root canal system in order to prevent reinfection (1). Although chemo-mechanical preparation reduces the number of microorganisms significantly, 40-60% of the root canals still remain positive for bacterial presence (2,3). The residual microorganisms are usually located in dentinal tubules, lateral canals and apical ramifications (4), thus preventing the healing of periradicular lesion, or decreasing the long-term success of root canal treatment (5,6). Therefore, the root canal obturation needs to entomb these residual bacteria and to prevent their access to periradicular tissues, and to block any other communications between the oral cavity and periradicular tissues. Besides, the antimicrobial activity of root canal sealers could contribute to further reduction of viable microorganisms (7,8).

The newest generation of root canal sealers are based on tricalcium silicate or calcium phosphate and they are called

Uvod

Cilj endodontskog liječenja jest uklanjanje intrakanalne infekcije i punjenje korijenskih kanala kako bi se spriječila ponovna infekcija tog područja (1). Iako kemijsko-mehanička obrada znatno smanjuje broj mikroorganizama, od 40 do 60 % korijenskih kanala ostaju pozitivni na mikroorganizme (2, 3). Zaostali mikroorganizmi najčešće se nalaze u dentinskim tubulusima, lateralnim kanalima i apikalnim deltama (4) te mogu spriječiti cijeljenje periradikularne lezije i rezultirati neuspjehom endodontskog liječenja (5, 6). Zato punjenje korijenskog kanala ima zadaću izolirati preostale mikroorganizme i spriječiti njihov put prema periradikularnim tkivima te onemogućiti bilo kakav oblik komunikacije usne šupljine s periradikularnim tkivima. Svrha punjenja korijenskih kanala jest daljnje smanjenje broja živih mikroorganizama (7, 8).

Najnovija generacija materijala za punjenje korijenskih kanala temelji se na trikalcijevu silikatu ili kalcijevu fosfatu

bioceramic sealers. They have attracted attention due to their alkaline pH, biocompatibility, bioactivity, non-toxicity, dimensional stability, sealing ability and potential to increace root strenght after obturation (9). Their antimicrobial efficacy has been evaluated in few past *in vitro* studies using different microbiological methods and obtaining different results (10-12). A recent study by Wang et al. (12) revealed same efficacy of Endosequence BC Sealer as AH Plus against *Enterococcus faecalis (E. faecalis)* in dentinal tubules after one day, seven days and 30 days of contact time. Another *in vitro* study reported superiority of the epoxy resin based sealer over a bioceramic sealer (13).

The aim of this study was to evaluate the short-term antimicrobial effect of three bioceramic root canal sealers and epoxy resin based sealer against 72 h old *E. faecalis* biofilm after 60 minutes of contact time.

Material and methods

Enterococcus faecalis cultivation

In this study, we used a wild type strain of *E. faecalis*, which had been isolated from a root canal of the lower first human molar with symptomatic chronic apical periodontitis. The bacteria were grown in brain heart infusion (BHI) broth at 4° C. Subsequently, few colonies were isolated and grown up on trypticase soy broth (TSB) at 37°C overnight. The density of the bacterial suspension was adjusted to 1.0 McFarland measured by a densitometer (Densimat, Bio Mérieux, Marcy l'Etoile, France).

For the evaluation of antimicrobial efficacy of bioceramic sealers and epoxy resin based sealer, we used the protocol with filter paper discs described by Barros et al. (14). The 10 µL of the *E. faecalis* suspension was grown on 81 filter paper discs (Whatman GmbH, Dassel, Germany), which were placed on Mitis Salivarius agar plates (Sigma-Aldrich, Taufkirchen, Germany). The paper discs with bacteria were incubated for 48 hours at 37°C and 100% of humidity. The discs were randomly choosen for scanning electron microscopy (SEM) in order to confirme biofilms growth. The selected discs were fixed in 2.5% glutaraldehyde (Sigma-Aldrich, Taufkirchen, Germany) for 24h and then dehydrated in an ascending ethanol solutions (60%, 70%, 80% and 96%), for 30 minutes in each. After 24 hours of drying on air, the samples were sputter-coated with gold and observed by the SEM (Tescan Vega TS5136LS, Tescan, Brno, Czech Republic).

Placement of sealers on filter discs

The filter discs with biofilms were divided into four experimental groups (n=17/each) according to the tested sealer used and the positive control group (n=10):

Group 1: Total Fill Bioceramic Sealer (TotalFill BC Sealer, FKG, Switzerland)

i naziva se biokeramičkim punilima. Pozornost su privukli zbog lužnatog pH, biokompatibilnosti, bioaktivnosti, netoksičnosti, dimenzijske stabilnosti, svojstva brtvljenja i potencijala za povećanje čvrstoće korijena nakon punjenja kanala (9). Njihova antimikrobna aktivnost ispitivana je u nekoliko istraživanja *in vitro* u kojima su se primijenile različite mikrobiološke metode pa su dobiveni i različiti rezultati (10 – 12). Nedavno istraživanje Wanga i suradnika (12) pokazalo je jednaku učinkovitost Endosequence BC Sealera i AH Plusa, kad je riječ o bakteriji *Enterococcus faecalis,* u dentinskim tubulusima nakon jedan, sedam i 30 dana kontakta. U drugom, pak, istraživanju *in vitro* istaknuta je bolja učinkovitost punila proizvedenog na temelju epoksidne smole u usporedbi s biokeramičkim punilom (13).

Cilj ovog istraživanja bio je ispitati kratkoročnu antimikrobnu učinkovitost triju biokeramičkoh punila i punila proizvedenog na temelju epoksidne smole na 72 sata stare biofilmove s *E. faecalis* nakon 60-minutnog kontakta.

Metode

Kreiranje biofilma s bakterijama *Enterococcus faecalis*

U ovom istraživanju koristili smo se divljim sojem bakterije *E. faecalis* izoliranim iz korijenskog kanala prvoga donjeg molara sa simptomatskim kroničnim apikalnim parodontitisom. Uzgojena je u moždano-srčanom bujonu (engl. *brain heart infusion* – BHI) na temperaturi od 4 °C. Nekoliko kolonija izolirano je uzgojeno i na mediju triptaza soj agara (engl. *trypticase soy broth* – TSB) preko noći na temperaturi od 37 °C. Gustoća bakterijske suspenzije podešena je denzitometrom (Densimat, Bio Mérieux, Marcy l'Etoile, Francuska) na 1,0 McFarland.

Korisili smo se protokolom s papirnatim filtar-diskovima opisanim u istraživanju Barrosa i suradnika (14), kako bismo ispitali antibakterijsku učinkovitost biokeramičkih punila i punila prozvedenog na temelju umjetne smole. Količina od 10 µL suspenzije *E. faecalis* postavljena je na 81 papirnati filtar-disk (Whatman GmbH, Dassel, Njemačka) te su zatim položeni na agarnu ploču Mitis Salivarius (Sigma-Aldrich, Taufkirchen, Njemačka). Papirnati diskovi s bakterijama inkubirani su 48 sati na 37 °C, u uvjetima 100-postotne vlažnosti. Nasumično odabrani diskovi pripremljeni su za promatranje pod pretražnim elektronskim mikroskopom (engl. *scanning electron microscopy* – SEM) kako bi se potvrdila prisutnost biofilmova.

Diskovi su tijekom 24 sata fiksirani u 2,5-postotnom glutaraldehidu (Sigma-Aldrich, Taufkirchen, Njemačka) te dehidrirani u sve većim koncentracijama otopine etanola (60 %, 70 %, 80 % i 96 %) 30 minuta u svakoj koncentraciji. Nakon 24-satnoga sušenja na zraku, uzorci su napareni zlatom i promatrani mikroskopom (Tescan Vega TS5136LS, Tescan, Brno, Republika Češka).

Postavljanje endodontskih punila na papirnate filtar-diskove

Papirnati filtar-diskovi s biofilmovima podijeljeni su u sljedeće četiri eksperimentalne skupine (n = 17/each), ovisno o ispitivanom punilu te u pozitivnu kontrolnu skupinu (n = 10):

Group 2: BioRoot Root Canal Sealer (BioRoot RCS, Septodont, Saint Maur Des Fosses, France)
Group 3: MTA Fillapex (Angelus, Londrina, PR, Brasil)
Group 4: Epoxy resin based sealer, AH Plus (Dentsply, Konstanz, Germany)

The tested sealers were prepared according to the manufacturers` instructions. Approximately, 40 µL of each freshly prepared sealer, which was measured by a spoon excavator (Premium instruments, New York, USA) of the same volume, was placed on the whole contaminated surface of filter disks. The sealer was spread over each disc using a single use sterile microbiological inoculating loop (Thermo Fisher Scientific, Waltham, MA, USA).

In the positive control group (n=10), the paper discs with biofilms remained without any sealers. In the negative control group, the sterile discs were not contaminated, but they were covered with the tested sealers (n= two samples for each sealer).

Microbiological analysis of antibacterial efficacy

After 60 minutes of contact time, the sealers were carefully removed from each disc using a sterile Heidelmann instrument (Hu-Friedy Mfg. Co., Frankfurt am Main, Germany). Some minor remnants remained on the surface of few discs. Each disc was placed in a sterile tube containing 1 ml od phosphate-buffered saline (PBS) and slightly agitated for 5 seconds (Vortex mixer X-HD, Sinosource, Guandgong, China) to remove unattached bacterial cells. Then, the discs were transferred to another sterile test tube, which contained 1 ml of PBS, and agitated for 30 seconds. After 8-fold serial dilutions, 10 µL of each dilution was grown on Mitis-Salivarius agar plate (Sigma-Aldrich, Taufkirchen, Germany) and incubated for 24 h on 37˚C and 100% humidity. After the incubation period, the colony forming units (CFUs) were counted and transformed into actual count based on the dilution factor.

Statistical analysis

The obtained data were analysed using the Mann-Whitney U test. All p-values lower than 0.05 were considered statistically significant. The program IBM SPSS Statistics version 23.0 (www.spss.com) was used.

Results

The SEM analysis confirmed the presence of biofilms of *E. faecalis* on filter discs (Figure 1, Figure 2).

All tested materials reduced the number of CFUs significantly compared with the positive controls (p<0.001) (Table 1). There was no statistically significant difference between the Total Fill BC Sealer and the AH Plus (p=0.386), nor between the BioRoot RCS and the MTA Fillapex (p=0.931) regarding the reduction of the CFUs. The Total Fill BC Sealer and the AH Plus showed greater antibacterial efficacy than the BioRoot RCS and the MTA Fillapex (p<0.001) (Figure 3).

Skupina 1: Total Fill Bioceramic Sealer (TotalFill BC Sealer, FKG, Švicarska)
Skupina 2: BioRoot Root Canal Sealer (BioRoot RCS, Septodont, Saint Maur Des Fosses, Francuska)
Skupina 3: MTA Fillapex (Angelus, Londrina, PR, Brazil)
Skupina 4: punilo proizvedeno na temelju epoksidne smole AH Plus (Dentsply, Konstanz, Njemačka).

Testirani materijali pripremljeni su prema uputama proizvođača. Otprilike 40 µL svakog svježe pripremljenog materijala, izmjerenog žličastim ekskavatorom (Premium instruments, New York, SAD) jednakog volumena, postavljeno je na kontaminiranu površinu papirnatoga filtar-diska. Punilo je na njegovu površinu razmazano mikrobiološkom ezom (Thermo Fisher Scientific, Waltham, MA, SAD).

Kao pozitivna kontrolna skupina (n = 10) korišteni su papirnati filtar-diskovi s biofilmovima koji nisu bili prekriveni punilima. Negativna kontrolna skupina bili su sterilni diskovi prekriveni eksperimentalnim punilima (n = dva uzorka za svako punilo).

Mikrobiološka analiza antibakterijske učinkovitosti materijala za punjenje

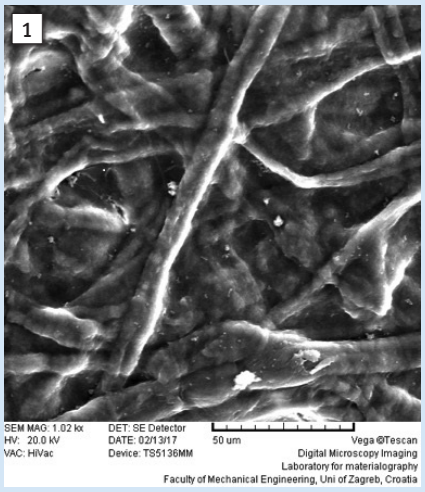
Nakon 60-minutnog kontakta punila su pažljivo uklonjena sa svakog diska sterilnom Heidelmannovom špatulom (Hu-Friedy Mfg. Co., Frankfurt na Majni, Njemačka). Manji ostatci punila ostali su na nekoliko diskova. Svaki disk stavljen je u sterilnu epruvetu koja je sadržavala 1 ml fosfatom puferirane fiziološke otopine i 5 sekunda je lagano protresen (Vortex mixer X-HD, Sinosource, Guandgong, Kina) kako bi se uklonile neadherirane bakterijske stanice. Zatim su diskovi prebačeni u drugu sterilnu epruvetu koja je sadržavala 1 ml fosfatom puferirane fiziološke otopine i treseni su 30 sekunda. Nakon 8 serijskih razrjeđenja, 10 µL suspenzije iz svakog razrjeđenja uzgojeno je na agarnim pločama Mitis-Salivarius (Sigma-Aldrich, Taufkirchen, Njemačka) i inkubirano 24 sata na 37 ˚C u uvjetima 100-postotne vlažnosti. Nakon inkubacije izbrojen je broj jedinica koje oblikuju kolonije (CFU-s) i izračunat konačni broj kolonija na temelju čimbenika razrjeđenja.

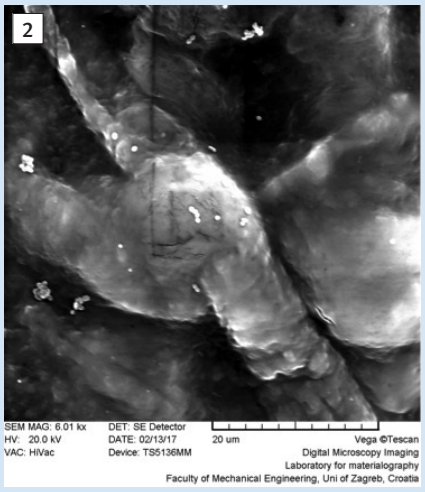
Statistička analiza

Dobiveni podatci analizirani su Mann-Whitneyjevim U testom. Sve p-vrijednosti niže od 0,05 razmatrane su kao statistički značajne. Za statističku analizu odabran je program IBM SPSS Statistics version 23,0 (www.spss.com).

Rezultati

Analiza SEM-om potvrdila je prisutnost biofilma s bakterijom *E. faecalis* na filtru papirnatih diskova (slika 1., slika 2.). Svi ispitivani materijali značajno su smanjili broj bakterija na filtar-diskovima u usporedbi s pozitivnom kontrolnom skupinom (p < 0,001) (tablica 1.). Nije bilo statistički značajne razlike između materijala TotalFill BC i AH Plus (p = 0,386) te između BioRoot RCS-a i MTA Fillapexa (p = 0,931). TotalFill BC i AH Plus bili su antibakterijski učinkovitiji negoli BioRoot RCS i MTA Fillapex (p < 0,001) (slika 3.).





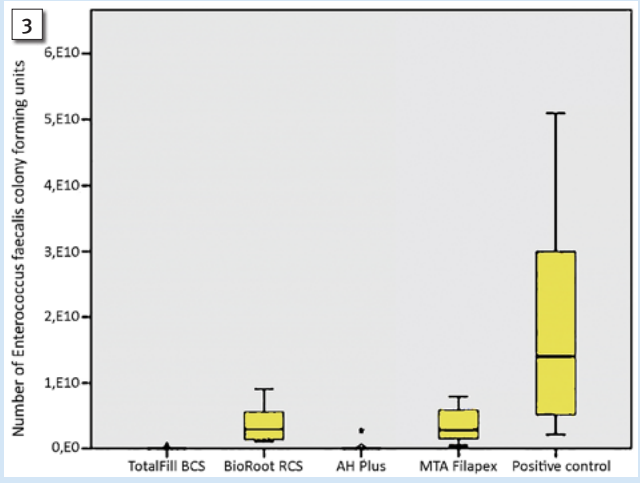


Figure 1 Scanning electron microscopy of *Enterococcus faecalis* colonization (1002x) of filter paper disk
Slika 1. SEM slika kolonizacije papirnatih filtar-diskova bakterijom *Enterococcus faecalis* (1002 x)
Figure 2 Scanning electron microscopy of *Enterococcus faecalis* colonization (6000x) of filter paper disk
Slika 2. SEM slika kolonizacije papirnatih filtar-diskova bakterijom *Enterococcus faecalis* (6000 x)
Figure 3 Number of *Enterococcus faecalis* colony forming units after the effect of each sealer and in the positive control group
Slika 3. Broj jedinica koje oblikuju kolonije bakterije *Enterococcus faecalis* nakon kontakta sa svakim punilom i pozitivnom kontrolnom skupinom

Table 1. Number of CFUs after 60 minutes of exposure to the tested root canal sealers and comparison to the positive control group
Tablica 1. Broj CFU-sa nakon 60-minutne izloženosti punilima i usporedba s pozitivnom kontrolnom skupinom

Groups • Skupine	Mean • Prosjek	SD	Minimum	Maximum • Maksimum	Percentiles • Percentili			Reduction compared to positive control • Smanjenje broja u odnosu prema pozitivnoj kontroli
					25th • 25 sati	50th (Median) • 50t sati (sred. vrijed.)	75th • 75 sati	
TotalFill BCS	3.72E+07	1.20E+08	1.00E+04	5.00E+08	1.00E+06	8.00E+06	1.00E+07	99.94%
BioRoot RCS	3.58E+09	2.56E+09	1.00E+09	9.00E+09	1.40E+09	2.90E+09	6.00E+09	79.29%
AH Plus	1.78E+08	6.51E+08	1.00E+05	2.70E+09	1.00E+06	1.00E+07	4.75E+07	99.93%
MTA Filapex	3.51E+09	2.50E+09	4.00E+08	7.90E+09	1.45E+09	2.80E+09	6.05E+09	80.00%
Positive control • Pozitivna kontrola	2.44E+10	2.75E+10	2.10E+09	9.00E+10	4.75E+09	1.40E+10	3.53E+10	

Discussion

Although all tested sealers showed significantly lower nuber of CFUs, there was no complete eradication of bacteria in any group. Firstly, these results can be explained due to the source of bacteria. In this study, wild-type strain of *E. faecalis* was used. Wild-type strains are less susceptible to sealers compared to most commonly used ATCC strain of *E. Faecalis*, and they have been recommended for the evaluation of antimicrobial efficacy of sealers (14). Also, *E. faecalis* has a significant role in etiology of persistent periradic-

Rasprava

Iako su svi testirani materijali pokazali da smanjuju broj jedinica koje formiraju kolonije (CFU-s), ni u jednoj skupini bakterije nisu potpuno eliminirane. Takvi se rezultati uglavnom vežu uz izvor bakterija. U ovom istraživanju korišten je divlji soj *E. faecalis.* Naime, divlji sojevi manje su osjetljivi u usporedbi s najčešće korištenim ATCC sojem bakterije *E. faecalis* na djelovanje punila te se preporučuju za ispitivanje njihove antimikrobne učinkovitosti (14). *E. faecalis* također je također važan u etiologiji perzistirajućih periradikular-

ular lesions (15) due to its virulence factors and ability to survive the conventional chemo-mechanical root canal procedures (16).

Secondly, the type of bacteria can affect antibacterial efficacy of sealers. Most previous studies evaluated the antibacterial efficacy of root canal sealers against planktonic bacteria (7, 13, 17). However, since planktonic bacteria do not represent a real clinical situation in infected root canals, the use of older bacterial biofilms which express greater resistance has been recommended (12,18,19). In this study, we used 48 hours old bacterial biofilm in a microbiological protocol described by Barros et al. (14). Therefore, the recommendation for further studies is to investigate the antibacterial efficacy of sealers on older biofilms.

Our finding of no complete eradication in all groups is cosistent with few previous studies (20,21). On the contrary, Zhang et al. (7) reported complete eradication of *E. faecalis* after application of both, the bioceramic root canal sealer (iRoot SP Sealer) (for 2 minutes) and the AH Plus (for 5 to 20 minutes) sealer. This result could be related to the planktonic species of bacteria used in suspension. These bacteria are more susceptible than biofilms (22).

The results of this study also showed similar antibacterial activity of the TotalFill BC Sealer and the epoxy resin based AH Plus against *E. faecalis* biofilms (eradication of more then 99.9%) after contact time of 60 minutes. The same conclusions have been reached in a recent study by Wang et al (12), in which no difference between the bioceramic sealer (Endosequence BC Sealer) and the AH Plus was found even after longer contact time of one day, seven days and 30 days using confocal microscopy evaluation. The antibacterial efficacy of bioceramic sealers is considered to be related to the release of Ca^{2+}ions and high pH (7,17,22). In another study by Nirupama et al (13), the AH Plus and the bioceramic iRoot SP sealers showed statistically significant antibacterial efficacy against *E. faecalis.* However, the AH Plus presented longer antibacterial effect (18 hours), probably due to its releasing of bisphenol-A-diglycidyl ether during polymerization (23). In this study, we evaluated the antimicrobial effect of the sealers only within 60 minutes of application since the direct contact test (DCT) on filter paper discs is solely intended for testing the short-term antimicrobial effect of the sealers (24). Namely, the DCT studies, where dentin is not preset, have shown that the sealers lose their antibacterial effect within a few hours or days from mixing (7, 24). Also, durations shorter than 60 minutes proved to be too short to affect resisting bacteria such as *E. faecalis* (25).

An interesting finding of this study is superior results of the Total Fill BC Sealer over the BioRoot RCS since both materials are bioceramics and of similar composition. The BioRoot RCS is the newest two-component root canal sealer. The powder component is composed of tricalcium silicate, zirconium dioxide and povidone, and the liquid is composed of water, calcium chloride and polycarboxylate. There have been only few studies published so far on its antibacterial activity (25,26). Poggio et al. (25) reported lower antibacterial activity of the BioRoot RCS compared with epoxy resin sealers against 18h old *E. faecalis*, which was explained due to its

nih lezija (15) jer sadržava čimbenike virulencije i svojstvo da preživi konvencionalnu kemomehaničku obradu korijenskih kanala (16).

Drugo, antibakterijska učinkovitost punila može ovisiti o starosnoj skupini bakterija. U većini dosadašnjih istraživanja ispitivana je antibakterijska učinkovitost punila na planktonskim bakterijskim stanicama (7, 13, 17). No te stanice ne pokazuju stvarnu situaciju u inficiranim korijenskim kanalima i zato se preporučuje korištenje starijih bakterijskih biofilmova koji su otporniji na antimikrobna sredstva (12, 18, 19). U ovom istraživanju koristili smo se 48 sati starim biofilmovima i mikrobiološkim protokolom opisanim u istraživanju Barrosa i suradnika (14). Preporuka je da se za daljnja istraživanja antibakterijske učinkovitosti upotrebljavaju punila starijih biofilmova.

Rezultati našeg istraživanja pokazuju nepotpunu eliminaciju bakterija u svim skupinama, što se može usporediti s dosadašnjim studijama (20, 21). Suprotno su pokazali Zhang i suradnici (7) u čijem su istraživanju biokeramički materijal Root SP Sealer (unutar 2 minute) i AH Plus (unutar 5 do 20 minuta) potpuno eliminitrali bakteriju *E. faecalis.* Takav se rezultat može objasniti činjenicom da su planktonske stanice osjetljivije od biofilmova na djelovanje antimikrobnih sredstava (22).

Naši rezultati također su pokazali sličnu antibakterijsku učinkovitost TotalFill BC punila i AH Plusa, materijala proizvedenog na temelju umjetne smole, kad je riječ o biofilmovima s *E. faecalis* (eliminacija 99,9 %) nakon 60-minutnog kontakta. Isto su zaključili i Wang i suradnici (13) u istraživanju u kojemu su s pomoću konfokalne laserske mikroskopije pokazali da nema razlike između biokeramičkog punila (Endosequence BC punila) i AH Plusa nakon duljih kontakata (jedan, sedam i 30 dana). Smatra se da je antibakterijska učinkovitost biokeramičkih punila povezana s otpuštanjem Ca^{2+}iona i visokih pH punila (7, 17, 22). U istraživanju Nirupame i suradnika (13), biokeramički materijali AH Plus i Root SP pokazali su značajnu antibakterijsku učinkovitost kad je riječ o *E. faecalis.* No AH Plus je bio dulje učinkovit (18 sati), vjerojatno zbog otpuštanja bisfenol-A-diglicidilnog etera tijekom polimerizacije (23). U ovom istraživanju ispitivali smo antimikrobnu učinkovitost punila unutar 60 minuta od postavljanja materijala, a koristili smo se direktnim kontaktnim testom (engl. *direct contact test* – DCT) zato što se DCT upotrebljava samo za ispitivanje kratkoročnog antimikrobnog učinka punila (24).

Naime, u istraživanjima u kojima se primijenio DCT, a nisu imala dentin kao podlogu, istaknuto je da punila gube antibakterijski učinak za nekoliko sati ili dana od miješanja (7, 24). I vrijeme kraće od 60 minuta pokazalo se prekratkim za početak djelovanja punila na bakterije poput *E. faecalis* (25).

Zanimljiv rezultat ovog istraživanja su superiorni rezultati dobiveni za Totalfill BC Sealera u usporedbi s BioRoot RCS-om, zato što su oba materijala biokeramička i imaju sličan sastav. BioRoot RCS najnovije je dvokomponentno punilo. Prah se sastoji od trikalcijeva silikata, cirkonijeva dioksida i povidona, a tekućina od vode, kalcijeva klorida i polikarboksilata. Dosad je objavljeno nekoliko istraživanja o njegovu antibakterijskom djelovanju (25, 26). Poggio i suradnici (25)

shorter working (minimum 10 min) and setting time (maximum 4 h) (26,27).

Another reason could be related to smaller homogeneity of the BioRoot RCS after hand mixing when compared with the "premixing" bioceramic sealers. In the most recent study of Arias-Moliz et al. (26), the BioRoot RCS showed better intratubular antibacterial efficacy compared with the MTA Fillapex and the AH Plus.

Different results of the same materials in the above mentioned studies could be explained due to different microbial tests used (10,25). The most commonly used microbial tests in this kind of studies are DCT and agar diffusion test (ADT) (17,25,28,29). ADT has been suitable for soluble materials but due to its limitations (dependence on diffusion and physical properties of tested materials, does not distinguish bacteriostatic or bactericidal effect of the material) (29) this test is no longer recommended (7). DCT is a quantitative and reproducible method to simulate the contact of microorganisms with endodontic sealers, providing information about bactericidal effect (7), thus being more reliable and relevant (9, 14).

In conclusion, Total Fill BCS and AH Plus presented similar antibacterial efficacy against *E. faeaclis*, which was superior compared to BioRoot RCS and MTA Fillapex.

Conflict of interest

None declared

opisali su slabiju antibakterijsku učinkovitost BioRoot RCS-a u usporedbi s punilom na bazi epoksidne smole na 18 sati stare biofilmove s bakterijom *E. faecalis*, što se tumači kraćim radnim vremenom (minimum 10 minuta) i vremenom stvrdnjavanja (maksimalno 4 sata) (26, 27). Drugi razlog mogao bi biti povezan s manjom homogenosti tog punila u usporedbi s onima već tvornički pripremljenima (engl. *premixing*. U najnovijem istraživanju Arias-Moliza i suradnika (26), BioRoot RCS postigao je bolju intratubularnu antibakterijsku učinkovitost u usporedbi s MTA Fillapexom i AH Plusom.

Različiti rezultati za iste materijale u navedenim istraživanjima mogu se objasniti korištenjem različitih mikrobioloških testova (10, 25). Najčešće korišteni testovi u takvoj vrsti istraživanja su DCT i agarni difuzijski test (ADT) (17, 25, 28, 29). ADT je odgovarajući test za ispitivanje topljivih materijala, ali zbog njegova ograničenja (ovisnost o difuzijskim i fizikalnim svojstvima ispitivanih materijala) ne može se ustanoviti je li riječ o bakteriostatskom ili baktericidnom učinku materijala (29) te se više ne preporučuje (7).

DCT je kvantitivna i ponovljiva metoda za oponašanje kontakta mikroorganizama s endodontskim materijalima i daje informaciju o baktericidnom učinku (7) pa je zato pouzdanija i relevantnija metoda (9, 14).

Zaključno, TotalFill BC Sealer i AH Plus pokazali su da slično antibakterijski djeluju na bakteriju *E. faeaclis*, i to bolje u usporedbi s BioRoot RCS-om i MTA Fillapexom.

Sukob Interesa

Autori nisu bili u sukobu interesa.

Sažetak

Ciljevi: Cilj istraživanja bio je ispitati antimikrobnu učinkovitost triju biokeramičkih materijala za punjenje korijenskog kanala na biofilm s bakterijama *E. faeaclis*. **Materijali i metode:** Bakterijski biofilm s enterekokima fekalis uzgojen je na papirnatim filtar-diskovima postavljenima na ploče s agarom. Nakon inkubacije diskovi su prekriveni s četirima različitim materijalima za punjenje korijenskih kanala: 1. jednokomponentnim biokeramičkim punilom (TotalFill BC Sealer); 2. dvokomponentnim biokeramičkim punilom (BioRoot RCS); 3. punilom proizvedenim na temelju mineralnog trioksidnog agregata (MTA Fillapex); 4. punilom proizvedenim na temelju epoksidne smole (AH Plus). Nakon kontaktnog 60-minutnog razdoblja materijali su uklonjeni, a diskovi uloženi u epruvete s puferiranom fiziološkom otopinom. Nakon serijskog razrjeđivanja, suspenzija je nasađena na ploče s agarom te su nakon 24 sata izbrojene narasle kolonije i određen njihov ukupan broj prema čimbeniku razrjeđenja. **Rezultati:** Nije bilo značajne razlike u antimikrobnoj učinkovitosti između TotalFill BC Sealera i AH Plusa ($p = 0,386$). Oba materijala pokazala su bolju antibakterijsku učinkovitost od BioRoot RCS-a i MTA Fillapexa ($p < 0,001$). **Zaključak:** Total Fill BC Sealer i AH Plus bolje antibakterijski djeluju od BioRoot RCS-a i MTA Fillapexa.

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