

Ispitivanje antibakterijskog svojstva endodontskih materijala

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Evaluation of antibacterial effect of endodontic materials

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KRATAK SADRŽAJ

Uvod. Usled nemogućnosti potpunog obesključavanja inficiranih kanala korena zuba tokom njihove mehaničko-medikamentozne obrade, od savremenih endodontskih materijala se očekuje da poseduju i odgovarajući antibakterijski efekat.

Cilj. Cilj ovog rada je bio da se u in vitro uslovima ispita antibakterijski efekat četiri savremena endodontska materijala: Acroseal, AH-Plus, Gutta Flow i Mineral Trioxide Agregate (MTA).

Materijal i metod. Antibakterijski potencijal testiranih materijala proveravan je na bakterijskom soju *Enterococcus faecalis* ATCC 29212, primenom agar difuznog testa.

Rezultati. Na osnovu dobijenih rezultata može se uočiti da su u sveže zamešenom stanju Acroseal i AH Plus ostvarili značajan antibakterijski efekat, dok Gutta Flow i MTA u ovom istraživanju nisu pokazali nikakvo antibakterijsko dejstvo. Kada su ispitivani materijali testirani u čvrstom stanju, nijedan od njih nije ispoljio antibakterijski efekat.

Zaključak. Testirani materijali za opturaciju kanala korena zuba, Acroseal i AH-Plus, u sveže zamešenom stanju imaju izvestan antibakterijski efekat, dok u čvrstom stanju ovaj efekat nije izražen.

Ključne reči: endodontski materijal, antibakterijsko svojstvo

SUMMARY

Introduction. Due to the inability to sterilize infected root canals during cleaning and shaping, contemporary endodontic materials are expected to possess certain antibacterial effect.

Aim. The aim of this study was to evaluate antibacterial effect of four contemporary endodontic materials: Acroseal, AH-Plus, Gutta Flow and Mineral Trioxide Aggregate (MTA) in vitro.

Materials and Methods. Antibacterial effect of tested materials was evaluated against bacterial species *Enterococcus faecalis* ATCC 29212 using the agar diffusion test.

Results. Based on the obtained results, it can be concluded that freshly mixed Acroseal and AH-Plus exhibited significant antibacterial effect while Gutta Flow and MTA did not present any antimicrobial potential in this study.

Conclusion. Endodontic sealers tested in this study, freshly mixed Acroseal and AH-Plus, possess certain antibacterial effect whereas in solid state this effect is not present.

Keywords: endodontic material, antibacterial effect

Složena anatomo-morfološka gradnja kanala korena zuba, njihova iregularnost, nepristupačnost i nemogućnost uspostavljanja apsolutno suvog polja rada u predelu apikalnih ramifikacija, čine fazu opturacije veoma složenom endodontskom procedurom. Situacija se dodatno kom-

Complex anatomo-morphological root canal system, its irregularity, inaccessibility and inability to maintain absolute dry working site in apical ramifications make obturation a very complex endodontic procedure. The situation is additionally complicated in cases of root canal

plikuje u slučaju infekcije korenskih kanala gde je, usled nemogućnosti potpunog obeskličavanja, realno očekivati i prisustvo rezidualne mikroflore^{1,2}. Mikrobiološki nalaz oboljenja pulpe je veoma raznovrstan. U većini slučajeva prisutna je mešovita anaerobno-aerobna infekcija, uz redovno prisustvo gljivica i protozoa. Mnogobrojna istraživanja su pokazala da inficirani korenski kanali najčešće sadrže između 10 i 50 različitih vrsta bakterija^{1,3}. *Enterococcus faecalis* spada u grupu fakultativno anaerobnih bakterija, i smatra se da je jedan od glavnih uzročnika neuspelih endodontskih terapija.⁴ *Enterococcus faecalis* je izuzetno otporan na klasičnu mehaničko-medikamentoznu terapiju, kao i na visok pH, zbog čega godinama može da preživi u dentinskim tubulima^{4,5}. Ovaj problem nameće potrebu da se pored obeskličavanja, koje se redovno obavlja tokom mehaničko-medikamentozne obrade korenskih kanala, antibakterijski efekat produži i izvesno vreme nakon faze opturacije endodontskog prostora. To je i razlog što se od jednog savremenog endodontskog materijala očekuje, da pored dobrih fizičko-hemijskih osobina, poseduje i odgovarajuća antibakterijska svojstva^{6,7}.

Cilj ovog rada je bio da se u *in vitro* uslovima, metodom agar difuzionog testa, ispita antibakterijski efekat četiri različita endodontska materijala.

Materijal i metod

Ispitivanje je obuhvatilo četiri savremena endodontska materijala koja se koriste za definitivno punjenje kanala korena zuba: Acroseal, AH Plus, Gutta Flow i Mineral Trioxide Agregate (MTA).

Antibakterijsko delovanje ovih preparata ispitano je agar difuzionom metodom (ADT), na standardnom bakterijskom soju *Enterococcus faecalis* ATCC 29212. Tokom eksperimenta bakterije su kultivisane na moždano-srčanom-infuzionom agaru (brain-heart infusion +BHI) obogaćenom sa 5% ovčije krvi. Podloga zasejana sa *E. faecalis* je inkubirana pod aerobnim uslovima na 35°C. Od kolonija ispitivanih bakterija nakon 24h inkubiranja pod gore navedenim uslovima na BHI agaru, napravljene su suspenzije u epruvetama sa sterilnim fiziološkim rastvorom, tako da je gustina svake pojedinačne bakterijske suspenzije odgovarala McFarland standardu 0,5. Suspenzija bakterija koja po gustini odgovara McFarland standardu 0,5 sadrži približno 10⁸ bakterija/ml. Ovako napravljene suspenzije su potom nanete sterilnim brisem na sveže razliven BHI agar obogaćen sa 5% ovčije krvi. Debljina razlivena podloge u plastičnim Petri šoljama je iznosila 4 mm. Zatim je sterilnim metalnim perforatorom, spoljašnjeg prečnika 5,5 mm napravljeno po četiri udubljenja u zasejanom BHI agar. Ovako formirana udubljenja zatim su napunjena sveže pripremljenim (ne starijim od 10 minuta) endodontskim materijalima. Priprema je urađena u skaldu sa uputstvima proizvođača. Petri šolje sa *E. faecalis* su inkubirane pod aerobnim uslovima. Nakon 48 h inkubiranja na 35°C izmeren je prečnik zone inhibicije rasta oko svakog udubljenja.

infections where residual microflora should be expected due to the inability to achieve complete disinfection.^{1,2} Microbiologic finding in pulp diseases is very diverse. In most cases, there is a combined anaerobic-aerobic infection with regular presence of fungi and protozoa. Numerous studies have shown that infected root canals most frequently contain 10 to 50 different bacterial species.¹⁻³ *Enterococcus faecalis* belongs to the group of facultative anaerobic bacteria and is considered one of the main causes of unsuccessful endodontic therapy.⁴ *Enterococcus faecalis* is extremely resistant to classic cleaning and shaping approach as well as high pH, and is able to survive in dentinal tubules for years.^{4,5} This problem poses the need to extend antibacterial effect after the obturation phase in addition to disinfection which is regularly performed during the cleaning and shaping phase. This is why contemporary endodontic material is expected to possess adequate antibacterial potential along its good physical and chemical properties.^{6,7}

The aim of this study was to *in vitro* assess antibacterial effect of four different endodontic materials using the agar diffusion test.

Materials and Methods

The study comprised four contemporary endodontic materials used for root canal obturation: Acroseal, AH-Plus, Gutta Flow and Mineral Trioxide Aggregate (MTA).

Antibacterial effect of these materials was assessed with the agar diffusion test (ADT) against standard bacterial species *E. faecalis* ATCC 29212. During the experiment, bacteria were cultivated on brain-heart infusion agar (BHI) enriched with 5% sheep blood. Substrates with *E. faecalis* were incubated in aerobic conditions at 35°C. Suspensions of bacterial colonies after 24 hour incubation were prepared with sterile saline in test tubes so the density of each bacterial suspension corresponded with McFarland standard 0.5. McFarland standard 0.5 meant that bacterial suspension contained cca 10⁸ bacteria/ml. The suspensions were put on fresh BHI agar enriched with 5% sheep blood. The thickness of fresh substrate in Petri dishes was 4 mm. A sterile metal perforator 5.5 mm in diameter was used to make four pits in BHI agar. These pits were filled with freshly prepared (not older than 10 minutes) endodontic materials. Materials were prepared according to manufacturers' instructions. Petri dishes with *E. faecalis* were incubated in aerobic conditions. After 48 hours of incubation at 35°C, the diameter of growth inhibition zone around each pit was measured.

Drugi deo ovog istraživanja obuhvatio je ispitivanje antibakterijskog efekta endodontskih materijala u čvrstom (vezanom) stanju. Metodologija je bila identična kao u slučaju sveže zamešanih materijala, izuzev što je ispitivanje antibakterijskog delovanja sprovedeno 48 h nakon pripreme endodontskih materijala.

Dobijene vrednosti zone inhibicije izražene u mm, obrađene su statistički (srednja vrednost,SD,CV) dok je za utvrđivanje statističke značajnosti korišćen Newman-Keulsov test.

Rezultati

Dobijene vrednosti prečnika zone inhibicije rasta bakterija na krvnom agaru prikazane su u tabeli 1.

Na osnovu dobijenih rezultata može se konstatovati da su Acroseal i AH Plus pokazali sličan antibakterijski efekat (slika 1 i 2). Gutta Flow i MTA nisu imali nikakvo antibakterijsko dejstvo kada su materijali ispitivani u sveže zamešanom stanju.

Testiranjem rezultata između grupa primenom Newman-Keulsovog testa, nije utvrđena statistički značajna razlika antibakterijskog potencijala između Acroseal-a i AH Plus $p > 0,05$.

Rezultati drugog dela ispitivanja, koji su se odnosili na endodontske materijale u vezanom (čvrstom) stanju, pokazali su da nijedan od četiri ispitivana materijala nije posedovao ni najmanji antibakterijski efekat, i ni u jednom slučaju nije registrovana zona inhibicije rasta bakterija.

The second part of this study was the evaluation of antibacterial effect of endodontic materials in solid state (after complete setting). Methodology was the same as described above. The only difference was that antibacterial effect was assessed 48 hours after material preparation.

Values for growth inhibition zones were in mm and statistically analyzed (mean, SD, CV). Newman-Keuls test was used to assess significance.

Results

Values for bacterial growth inhibition zones on blood agar are presented in table 1.

Based on the obtained results, it can be noted that Acroseal and AH-Plus exhibited similar antibacterial effect (Figures 1 and 2). Freshly mixed Gutta Flow and MTA did not have any antibacterial effect.

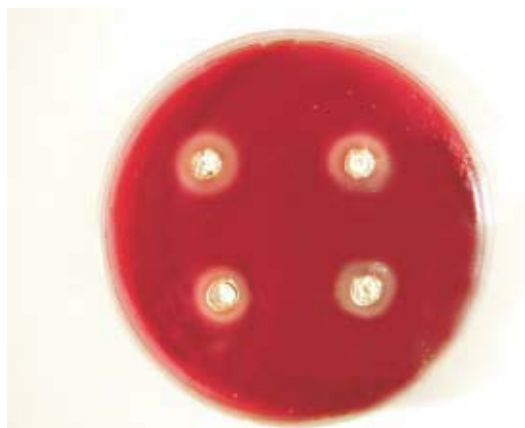
Newman-Keuls test revealed there was no significant difference in antibacterial potential of Acroseal and AH-Plus ($p > 0.05$)

The second part of the study, which referred to endodontic materials after setting, showed that none of four tested materials had even the slightest antibacterial effect. Growth inhibition zones were not detected in any case.

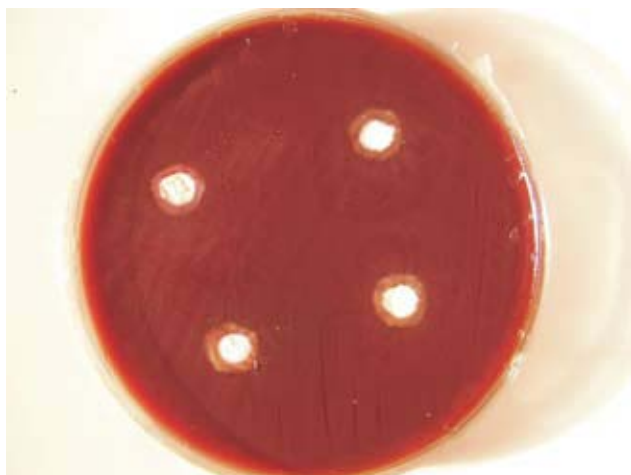
Tabela 1. Prosečne zone inhibicije rasta bakterija (SD,CV)

Table 1. Mean values of growth inhibition zones of bacteria (SD,CV)

MATERIJAL	N	X	SD	CV(%)	min-max
AH PLUS	4	10,00	1,13	11,3	9-11
ACROSEAL	4	11,11	1,48	12,8	10-12
GUTTA FLOW	4	0	0	0	0
MTA	4	0	0	0	0



Slika 1. Zone inhibicije rasta Acroseal-a u BHI agaru
Figure 1. Growth inhibition zones Acroseal in BHI agar



Slika 2. Zone inhibicije rasta AH-Plus-a u BHI agaru
Figure 2. Growth inhibition zones AH-Plus in BHI agar

Diskusija

I pored toga što je ADT- metod prihvaćen kao osnovni model u ispitivanjima antibakterijske aktivnosti stomatoloških materijala, ne treba zaboraviti na to da je na osnovu ovog metoda teško utvrditi da li određeni materijal poseduje baktericidno ili samo bakteriostatičko dejstvo^{7,8}. Takođe se, na osnovu ovog testa ne dobijaju adekvatni podaci o dužini trajanja antibakterijskog efekta.

Hronične periapiksne inflamatorne lezije izazivaju različite vrste mikroorganizama, pri čemu broj i vrsta varira od slučaja do slučaja. Mnogobrojna mikrobiološka istraživanja utvrdila su da u nastanku apikalnih parodontitisa primarnu ulogu imaju striktno anaerobne bakterije, kao i neke vrste fakultativno anaerobnih bakterija kao što su streptokoke^{1,3}. Kompletna eliminacija mikroorganizama iz kanala korena zuba predstavlja osnovni cilj svake endodontske terapije inficiranih kanala^{8,9}. Ovaj cilj se postiže kroz adekvatnu mehaničko-medikamentoznu obradu kanala korena zuba, kao i uz naknadnu hermetičnu opturaciju endodontskog prostora. Međutim, i pored pažljivog tretmana i oblikovanja kanala korena zuba bakterije mogu zaostati u dentinskim tubulima, lateralnim kanalima i apikalnoj delti, te mogu ponovo rekolonizovati kanal korena zuba. Pored toga bakterije mogu prodreti u zatvoreni kanal korena zuba, ukoliko kanal nije adekvatno i trodimenzionalno opturisan.

Ove bakterije mogu naknadno izazvati upale apikalnog parodontijuma, pa je stoga poželjno da endodontski materijali poseduju odgovarajuća antibakterijska svojstva. Ovako nastale apikalne inflamatorne lezije najčešće izaziva *Enterococcus faecalis*, dok striktno anaerobne bakterije imaju manji značaj⁴. Brojna istraživanja su pokazala da je *E. faecalis* veoma otporan na standardnu mehaničko-medikamentoznu terapiju, i da je osetljiv jedino na dejstvo hlorheksidina^{4,5}.

Acroseal je cementna smola na bazi kalcijum hidrok-sida, epoksi-bis-fenol smole, meteneamina i enoksolona¹¹. Acroseal je u ovom ispitivanju pokazao naj snažnije antibakterijsko delovanje koje se može pripisati uticaju kalci-

Discussion

Even though ADT has been adopted as the basic model in studying antibacterial activity of dental materials, it must be noted that it is difficult to differentiate whether certain material possesses bactericidal or only bacteriostatic effect based on this method.^{7,8} Furthermore, adequate data on the longevity of antibacterial effect cannot be gathered using this test.

Chronic periapical inflammatory lesions are caused by different types of microorganisms with varying numbers and species from case to case. Numerous microbiologic studies have found that strict anaerobic bacteria have a primary role in the development of apical periodontitis as well as some forms of facultative anaerobic bacteria like streptococci.^{1,3} Complete elimination of microorganisms from the root canal is the primary goal of any endodontic therapy of infected canals.^{8,9} This is achieved through adequate cleaning and shaping of the root canal followed by hermetic obturation of endodontic space. However, despite a careful treatment and root canal shaping, bacteria may remain in dentinal tubules, lateral canals and apical delta with the potential of re-colonization of the root canal. Additionally, bacteria may penetrate into an obturated root canal if the seal is not adequate and three-dimensional.

Subsequently, bacteria may cause the inflammation of apical periodontium and, therefore, it is desirable that endodontic materials possess certain antibacterial effect. Such apical inflammatory lesions are most often caused by *E. faecalis*, while strict anaerobic bacteria are of less importance.⁴ Numerous studies have shown that *E. faecalis* is very resistant to standard cleaning and shaping therapy and that it is sensitive only to the effect of chlorhexidine.^{4,5}

Acroseal is a cement resin based on calcium-hydroxide, epoxi-bisphenol resin, metenamine and enoxolon.¹¹ In the present study, Acroseal showed the most powerful

jum hidroksida. Zone inhibicije rasta su bile najveće oko udubljenja ispunjenih Acroseal-om, što ukazuje na dobru difuziju kao i na snažan antibakterijski potencijal ovog endodontskog materijala. Acroseal pripada novijoj grupi endodontskih pasti i u dostupnoj literaturi nema podataka o njegovom antibakterijskom delovanju.

AH Plus je dvokomponentna pasta koja bazira na epoksiaminskoj smoli¹¹. U istraživanju Mickel-a i sar. nije pokazao antimikrobni efekat prema *E. faecalis*¹⁰. Međutim, u ovom radu, kao i u istraživanjima drugih autora sveže pripremljeni AH Plus je pokazao izuzetno delovanje prema *E. faecalis*^{4,12}. Cohen i sar. dokazali su da AH-Plus otpušta male količine formaldehida (3,9 ppm), što bi moglo uticati na ispoljavanje njegovog antibakterijskog efekta¹⁵.

MTA je endodontski cement sastavljen od hidrok-silnih čestica više oksida, pri čemu glavnu komponentu praha čine CaO, SiO₂, Al₂O₃ i Bi₂O₃¹⁵. MTA u ovom eksperimentu nije pokazao antibakterijsko delovanje prema *E. faecalis*, što potvrđuju i nalazi Torabinejada i sar. kao i Estrele i sar.^{17,18}. MTA je slabo rastvorljiv zbog čega slabo difunduje pa se time može protumačiti odsustvo antimikrobne aktivnosti prema *E. faecalis* u agar difuzionom metodu koji smo koristili.

Gutta Flow je savremeni endodontski materijal čiju osnovu čini polivinilsiloksan¹¹. U ovom ispitivanju slično kao i u istraživanju Cobankara i sar. Gutta Flow nije ispoljio antibakterijski efekat na *E. faecalis*²⁰.

Ranija ispitivanja ukazivala su na činjenicu da antibakterijska svojstva endodontskih materijala dolaze do izražaja samo dok se oni nalaze u plastičnom stanju, da bi se nakon definitivnog stvrdnjavanja taj efekat potpuno gubio^{7,8}. I rezultati ovog rada to potvrđuju, budući da ni u jednom slučaju endodontski materijali primenjeni u čvrstom stanju nisu pokazali zonu inhibicije rasta bakterija u krvnom agaru.

Na kraju iako rezultate *in vitro* ispitivanja treba prihvatiti sa određenom dozom opreza, mora se istaći da oni mogu da posluže kao dobra osnova za dalja klinička ispitivanja.

Zaključak

Ispitivanja antibakterijskog efekta endodontskih materijala, u sveže zamešanom stanju, su pokazala da Acroseal i AH Plus imaju izvestan antibakterijski efekat prema *Enterococcus faecalis*-u, a Gutta Flow i MTA ga ne poseduju. Na osnovu ovog bakteriološkog istraživanja može se zaključiti da ni jedan od ispitivanih endodontskih materijala u čvrstom stanju, nije pokazao inhibitorno dejstvo na rast *E. faecalis*-a.

antibacterial potential that can be attributed to calcium-hydroxide. Growth inhibition zones were greatest around Acroseal-filled pits suggesting good diffusion and significant antibacterial potential of this material. Acroseal belongs to the group of newer endodontic sealers with no literature data on its antibacterial activity.

AH-Plus is a two component paste based on epoxy-amine resin.¹¹ In Mickel et al. study, it did not show any antibacterial effect against *E. faecalis*.¹⁰ However, in the present study, as well as other studies, freshly mixed AH-Plus exhibited powerful activity against *E. faecalis*.^{4,12} Cohen et al. have found that AH-Plus releases small quantities of formaldehyde (3.9 ppm) which may be accounted for its antibacterial effect.¹³

MTA is an endodontic cement consisting of hydroxyl particles of several oxides with main powder components in the form of CaO, SiO₂, Al₂O₃ and Bi₂O₃.¹³ In this experiment, MTA did not show antibacterial activity against *E. faecalis* as confirmed by Torabinejad et al. and Estrela et al.^{15,16} MTA is poorly soluble and the lack of antibacterial activity against *E. faecalis* may be due to its poor diffusivity.

Gutta Flow is a contemporary endodontic material based on polyvinylsiloxane.¹¹ In this study, similarly to the study of Cobankar et al, Gutta Flow did not present antibacterial effect against *E. faecalis*.¹⁸

Previous studies have suggested that antibacterial property of endodontic materials is evident only in the plastic state and disappears after complete setting.^{7,8} The present results confirm this as in any case no bacterial growth inhibition zone was observed after material setting.

Even though the results of this *in vitro* study should be accepted with certain caution, they could serve as a good reference point for further clinical investigation.

Conclusion

The evaluation of antibacterial effect of freshly mixed endodontic materials has revealed that Acroseal and AH-Plus possess certain antibacterial effect against *Enterococcus faecalis*, while Gutta Flow and MTA do not. Based on the present microbiologic study, it can be concluded that none of tested endodontic materials in the solid state possesses inhibitory activity against the growth of *E. faecalis*.

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