

Antimikrobna aktivnost materijala za trajnu opturaciju kanala korena zuba

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Antimicrobial activity of root canal sealers

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KRATAK SADRŽAJ

Svi materijali za definitivno punjenje kanala korena bi trebalo da imaju antimikrobna svojstva kako bi posle opturacije mogli delovati na eventualno zaostale mikroorganizme.

Cilj: Cilj ovog istraživanja je bio da se ispita antimikrobni efekat pet materijala za opturaciju kanala: AH 26 paste na bazi epoksi smole, Apexit-a, paste na bazi kalcijum hidroksida, materijala na bazi cink oksid eugenola Endomethasone-a i Tubliseal-a i glas jonomer cementa Ketac Endo Aplicap.

Materijal i metod: Antimikrobna aktivnost je testirana na mikroorganizmima *S.mutans* OMZ 70C i *L.casei* ATCC 27773 primenom ADT testa (Agar Diffusion Inhibitory test) na TYC SB, krvnom i MRS agaru. Na agar pločama, u udubljena prečnika 4 mm je ubačena sveže zamešana pasta ili cement. Zasejane agar ploče su inkubovane su anaerobno na 37° C u GAS PAK sistemu u trajanju 24 h. Antibakterijski efekat je procenjen na osnovu veličine prečnika zone inhibicije rasta mikroorganizama oko ispitivanih materijala. Urađena su po 3 merenja za svaki materijal, a vrednosti su izražene u milimetrima. Statistička analiza urađena je Mann Whitney testom.

Rezultati: Dobijeni rezultati su potvrdili da najjači antimikrobni efekat poseduju paste na bazi epoksi smole i paste na bazi cink oksid eugenola. Kalcijum hidroksidne paste i glas jonomer cement su pokazale statistički značajno slabije antibakterijsko dejstvo u odnosu na AH 26, Endomethasone i Tubliseal.

Zaključak: Najjači antibakterijski efekat je pokazala pasta na bazi epoksi smola AH 26 na oba testirana mikroorganizma.

Ključne reči : paste za opturaciju kanala, antimikrobni efekat

SUMMARY

All root canal sealers should have antimicrobial activity so that they could act against any remaining bacteria after root canal obturation. **The aim** of this study was to evaluate the antimicrobial activity of five root canal sealers: AH26, a resin based paste; Apexit, calcium hydroxide based paste; Endomethasone and Tubliseal, zinc oxide eugenol based materials and Ketac Endo Aplicap, glass ionomer based sealer.

Materials And Methods: Antimicrobial activity was tested against *S. mutans* 70C and *L.casei* ATCC 27773 using ADT (agar diffusion inhibitory test) on TYC SB, blood and MRS agars. Freshly prepared paste or cement was placed in grooves of 4 mm in diameter in agar plates. Prepared agar plates were incubated at 37°C in GAS PAC system for 24 hours. The antimicrobial effect was determined according to the diameter of growth inhibition zone around tested materials. Three measurements were done for each material and values were recorded in millimeters. Statistical analysis was done using Mann Whitney test.

The **results** confirmed that epoxy resin and zinc oxide eugenol based sealers had the greatest antimicrobial effect. Calcium hydroxide and glass ionomer based sealers showed significantly lower antimicrobial activity compared to AH26, Endomethasone and Tubliseal.

Conclusion: The greatest antimicrobial activity was found for epoxy resin based sealer (AH26) for both tested microorganisms.

Keywords: root canal sealers, antimicrobial effect

Osnovni cilj endodontske terapije je prevencija i kontrola infekcije u kanalu korena. Početna kontrola uspostavlja se biomehaničkom preparacijom. Naime biomehanička preparacija kanala, irigacija, intrakanalna medikacija kao

The main goal of endodontic therapy is prevention and control of root canal infection. The initial control is set by biomechanical preparation. Biomechanical preparation of a root canal, irrigation, intracanal medication

i uklanjanje razmaznog sloja najvećim delom uklanjaju mikroorganizme i njihove produkte iz inficiranog kanala.

Trodimenziona hermetična opturacija eliminiše prazne prostore u kanalu koji predstavljaju potencijalni izvor infekcije i sprečava mikropropustljivost tkivne tečnosti i mikroorganizama iz periapiksa u kanal,(1). Pored toga materijali za definitivno kanalno punjenje bi trebalo da poseduju antimikrobno dejstvo. Opturacijom kanala korena blokiraju se zaostali mikroorganizmi u dentinskim kanalčićima, a produženim antimikrobnim dejstvom paste sprečavaju rast i razvoj mikroorganizama,(2,3). Time se sprečava reinfekcija i omogućava reparacija u predelu periapiksnog tkiva što značajno utiče na povoljan ishod endodontske terapije.

Obzirom na značaj ovog problema brojni autori i studije su proučavali antimikrobni efekat materijala za definitivno kanalno punjenje kao jedne od važnih bioloških osobina, (4-10).

Ispitujući antimikrobni efekat materijala za opturaciju ADT i direktnim kontakt testom (DCT), Cobankara i sar nalaze slabiji efekat glas jonomer cementa Ketac Endo u odnosu na paste na bazi smola i cink oksid eugenola. Autori napominju da na rezultate antimikrobnog efekta utiču vrsta testa, vreme testiranja i pojedine komponente iz testiranih materijala (5). Fuss i sar proveravaju materijale na bazi kalcijum hidroksida (Sealapex i CRCS) i cink oksid eugenolni Roth-ov cement i nalaze da oba materijala pokazuju bakteriostatski efekat već posle 7 dana. Roth-ov cement ima jači antibakterijski potencijal u sveže zamešanom stanju, da bi posle 7 dana Sealapex pokazao jače antimikrobno dejstvo, (6). Jukić S i sar primenjuju Ames test u ispitivanju mutagenog potencijala pasta na bazi smole na Salmonellu. Autori beleže jaču mutagenu aktivnost sveže zamešane paste AH 26 u trajanju i do mesec dana. Dok je mutageni potencijal AH Plus (sveže zamešanog) znatno slabiji u odnosu na AH 26, da bi nestao po vezivanju paste,(7). Shalvav, Fuss u svojim istraživanjima ukazuju na jak antibakterijski efekat paste na bazi smole (AH 26), (8).

Cilj ovog rada je bio da se provere antimikrobna aktivnost četiri različita materijala za definitivnu opturaciju kanala korena.

Materijal i metod

U ispitivanjima su obuhvaćene paste za definitivno kanalno punjenje: AH 26, pasta na bazi epoksi smole, Apexit, paste na bazi kalcijum hidroksida, materijali na bazi cink oksid eugenola Endomethasone i Tubliseal i glas jonomer cement Ketac Endo Aplicap, Tabela 1,2.

Antimikrobna aktivnost je testirana na mikroorganizmima S.mutans OMZ 70C i L.casei ATCC 27773 primenom ADT testa (Agar Diffusion Inhibitory test). S. mutans je zasejan na TYC SB i na krvnom agaru, a L.casei na MRS i krvnom agaru. Na pripremljenih 12 agar ploča vrhom sterilnog staklenog štapića pravljena su udubljenja

as well as smear layer removal eliminate the greatest amount of microorganisms and their by-products from the canal.

Three-dimensional hermetic obturation eliminates empty spaces in the root canal that present potential infection sources and prevents microleakage of tissue fluid and microorganisms from periapex into the canal (1). Apart from that, root canal sealers should possess antimicrobial activity. Obturating root canal, the remaining microorganisms in dentinal tubules are blocked. With their antimicrobial activity sealers prevent microbial growth (2,3). Thus, reinfection is disabled and reparation in periapical tissue enabled which is of great importance for the success of endodontic therapy.

Regarding the importance of this problem, many authors investigated antimicrobial activity of root canal sealers, as one of important biological properties. (4-10)

Evaluating antimicrobial effect of root canal sealers using ADT and direct contact test (DCT), Cobankara et al. found lower effect for glass ionomer sealer Ketac Endo compared to resin based and eugenol based sealers. Authors stressed that type and duration of tests and material components affect the results of antimicrobial activity.(5) Fuss et al. tested calcium-hydroxide based sealers (Sealapex and CRCS) and eugenol based Roth cement and found that all materials exhibited bacteriostatic effect after 7 days. Roth cement had stronger antibacterial potential when freshly mixed, whereas after 7 days Sealapex showed better antimicrobial effect.(6) Jukic et al conducted Ames test to determine the mutagenic potential of resin based sealers against Salmonella spp. Authors reported stronger mutagenic activity of freshly mixed AH26 up to a month and a half, while mutagenic effect of freshly mixed AH Plus is much lower and disappears upon setting.(7) Shalvav and Fuss reported strong antibacterial effect of resin based sealer AH 26.(8)

The aim of this study was to evaluate the antimicrobial effect of four different types of root canal sealers.

Materials and Methods

Four types of root canal sealers were included in the study: resin based AH26, calcium-hydroxide based Apexit, eugenol based Endomethasone and Tubliseal and glass ionomer based Ketac Endo Aplicap. (Tables 1 and 2)

The antimicrobial activity was tested against S. mutans OMZ 70C and L.casei ATCC 27773 using ADT test (Agar Diffusion Inhibitory test). S. mutans was colonized on TYC SB and blood agar and L. casei on MRS and blood agar. Grooves of 4 mm in diameter were made with the tip of a sterile glass stick on already prepared 12

prečnika 4 mm u koje je ubačen sveže zamešana pasta ili cement za trajnu opturaciju kanala. Zasejane agar ploče sa sveže zamešanim materijalom inkubovane su anaerobno na 37° C u GAS PAK sistemu u trajanju 24 h. Antibakterijski efekat je procenjen na osnovu veličine prečnika zone inhibicije rasta mikroorganizama oko ispitivanih materijala. Uradjena su po 3 merenja za svaki materijal, a vrednosti su izražene u milimetrima.

Statistička analiza urađena je Mann Witney testom.

Tabela 1 Testirane paste (cement) za definitivnu opturaciju
Table 1. Tested sealers

Vrsta materijala	Proizvodjač	Br. Uzoraka	
		I grupa	II grupa
1. Ketac-Endo Aplicap	ESPE, GmbH, See feld Oberbay Germany	20	16
2. Apexit	Vivadent, Liechtenstein	19	14
3. Tubliseal	Kerr, Manufacturing Co, Rumulus MI	20	15
4. Endomethasone	Septodonte Paris, France	18	14
5. AH 26	De tray Ag Zurich Switzerland	20	15
UKUPNO:		97	74

a) Za proveru adherentnosti metodom SEM korišćeno je 30 sveže ekstrahovanih prednje maksilarnih zuba podeljenih u pet grupa po 6 uzoraka, zavisno od vrste materijala za punjenje kanala. Po dva uzorka iz svake grupe su ostala izdvojena sa prisutnim razmaznim slojem, a na 4 uzorka razmazni sloj je bio uklonjen pomoću 25% vodenog rastvora taninske kiseline (tabela 2).

b) Materijal za ispitivanje antibakterijskog svojstva bio je:

- **test mikroorganizmi:** - Streptococcus Mutans OMZ 70 C
- Lactobacillus Cassei ATCC 27773

- **hranljivi agar:** - TYC-SB agar
- krvni agar
- MRS agar

- sveže zamešani testirani materijal za punjenje kanala (tabela 1)
- izdvojeni elementi dvokomponentnih materijala, prah i tečnost (tabela 2)

Pojedinačne komponente bile su izdvojene iz: Apexit-a, Tubliseal-a, Endomethasone-a i AH 26 (tabela 3):

- "a" komponenta koju sačinjavaju prah ili aktivna baza

- "b" komponentu čini tečnost ili katalizator

agar plates and freshly mixed root canal sealers (paste or cement) were placed in them. Colonized agar plates containing root canal sealers were incubated in anaerobic conditions at 37°C in the GAS PAC system for 24 hours. The antimicrobial effect was determined according to the diameter of growth inhibition zone around tested materials. Three measurements were done for each material and values were recorded in millimeters. Statistical analysis was done using Mann Whitney test.

Tabela 2 Sastav testiranih pasta (cementa)
Table 2. Sealer composition

Materijal	Sadržaj	%		
Apexit	Baza	Kalcijum hidroksid,	32	
		Kolofonij	32	
		Silikon dioksid	8	
		Kalcijum oksid	6	
		Trikalcijum fosfat	4	
		Zn stearat	2	
		ZnO	6	
		Polidimetilsiloksan	3	
		Aktivator	Trimetilheksadisalicilat	25
			Bizmut karbonat	18
			Bizmut oksid	2
Silikon dioksid	15			
1,3 butan disalicilat	11			
Tubliseal	Baza	Kolofoni	5	
		Trikalcijum fosfat	5	
		Zn stearat	1	
		ZnO	40	
		Ba sulfat	2,75	
		Ulje smole	25	
		Timol jodid	7,5	
		Modifikatori	2	
		Aktivator	Ulje karanfilića	
			Canada balsam	
		Endomethasone	Prah	Deksametazon
Hidrokortizon acetat	1			
Ti jodid	25			
Paraformaldehid	2,2			
Tečnost		Rengenkontrast	72	
		Eugenol		
AH 26	Prah	Srebro prah	10	
		Bi oksid	60	
		Heksametilentetramin	25	
		Ti oksid	5	
Tečnost		Epoksisibifenol		
Ketac Endo Aplicap	Prah	Kalcijum, aluminijum lantan, fluoro silikatne čestice stakla, kalcijum volfram, pigmenti		
Tečnost		Akrična kiselina /maleinska kiselina, kopolimeri, tartarna kiselina, voda		

Rezultati

Dobijeni rezultati bakterioloških ispitivanja prikazani su na Tabelama 3,4,5,6 i na Slici 1. Registrovane su prosečne vrednosti prečnika zone inhibicije rasta bakterija na ispitivanim odabranim podlogama oko svakog materijala na testiranim mikroorganizmima..

Najveću antibakterijsku aktivnost na *S.mutans* pokazao je AH 26 sa zonom inhibicije rasta mikroorganizama od 20mm na krvnom i 26,23 na TYC- SB agaru. Potom sledi Tubliseal i Endomethasone sa manjim efektom na krvnom (8,66 i 9,66mm) i većim na TYC-SB agaru (15,00 i 13,00). Ketac Endo Aplicap je dao umereno i ujednačeno antibakterijsko dejstvo na *S.mutans* na oba agara. Prosečna vrednost prečnika z.inhibicije rasta bakterije na krvnom agaru iznosila je 11,83 mm, a na TYC-SB agaru 10,33 mm. Apexit nije pokazao antibakterijski efekat na *S.mutans* ni na jednoj podlozi, (Tabela 3).

Results

The results are presented in Tables 3,4,5 and 6 and in Figure 1. The average diameters of growth inhibition zones were recorded around each tested material.

The strongest antimicrobial activity against *S. mutans* was exhibited by AH26, producing a growth inhibition zone of 20 mm on blood agar and 26.23 mm on TYC-SB agar. This was followed by Tubliseal and Endomethasone with lower effect on blood (8.66 and 9.66 mm respectively) than on TYC-SB agar (15 mm and 13 mm, respectively). Ketac Endo Aplicap produced moderate effect against *S. mutans* on both agar types. The average growth inhibition zone was 11.83 mm on blood agar and 10.33 mm on TYC-SB agar. Apexit exhibited no antibacterial activity on either types of agar. (Table 3)



Slika 1 Antimikrobno dejstvo sveže testiranih materijala na *S.mutans* na TYC-SB agaru :
1- AH 26 2- Endomethasone 3- Tubliseal
4- Apexit 5- Ketac Endo Aplicap

Figure 1. Antimicrobial activity of freshly prepared sealers against *S. mutans* on TYC-SB agar.
1- AH 26 2- Endomethasone 3- Tubliseal 4- Apexit 5- Ketac Endo Aplicap

Tabela 3 Prosečne vrednosti prečnika zone inhibicije rasta (u mm)

Table 3. Average diameter values for growth inhibition zone (in mm)

Red. br.	Vrsta materijala	Streptococcus Mutans	
		Krvni agar	TYC-SB agar
1.	Ketac-Endo Aplicap	11,83 ± 0,3	10,33 ± 1,5
2.	Apexit	0,00	0,00
3.	Endomethasone	8,66 ± 2,3	13,00 ± 1,5
4.	Tubliseal	9,66 ± 1,5	15,00 ± 0,2
5.	AH 26	20,00 ± 1,00	26,33 ± 0,6

Statistički je potvrđeno da sveže zamešan AH 26 ima najjače antimikrobno dejstvo, na podlogama gde je kultivisan *S.mutans*, u odnosu na ostale testirane materijale, (Tabela 4).

U okviru mikrobioloških ispitivanja provereno je i dejstvo sveže zamešanih materijala na *L.casei* na dve hranljive podloge, krvnom i MRS agaru, (Tabela 5).

Najveća prosečna vrednost *z.inhibicije rasta* na *L.casei* tj.najjači antimikrobni efekat zabeležen je kod AH 26 posebno na MRS-u (37,3 mm), kao i na krvnom agaru (18,3 mm). Slede materijali na bazi cink oksid eugenola, Tubliseal i Endomethasone i to na krvnom agaru (22,7 i 20 mm). Nešto slabiji antibakterijski efekat ove paste pokazuje na *L.casei* na MRS agaru (11,7 i 10,7 mm). Potom sledi Apexit sa ujednačenim antibakterijskim efektima na *L.casei* na obe podloge (15,0 na krvnom i 16,7 mm na MRS agaru). Ketac Endo je pokazao ujednačen i slabiji efekat na *L casei* na oba agara, (Tabela 5).

Tabela 5 Prosečna vrednost zone inhibicije rasta (u mm)

Table 5. Average values of growth inhibition zones (in mm)

Red. br.	Vrsta materijala	Lactobacillus casei	
		Krvni agar	MRS agar
1.	Ketac-Endo Aplicap	9,7 ± 0,6	13,7 ± 2,3
2.	Apexit	15,0 ± 0,0	16,7 ± 1,5
3.	Endomethasone	20,7 ± 5,1	10,7 ± 1,1
4.	Tubliseal	22,7 ± 1,5	11,7 ± 2,1
5.	AH 26	18,3 ± 2,1	37,3 ± 7,3

Tabela 6 Statistička značajnost razlika u prosečnim vrednostima *z.inhibicije rasta L.casei*Table 6. Statistical significance among tested sealers in regard to growth inhibition zone of *L. casei*

KRVNI AGAR					
Materijal	Ketac-Endo	Apexit	Endomethasone	Tubliseal	AH 26
Ketac-Endo		p<0,001**	p>0,05	p<0,01**	p<0,05*
Apexit	15,92		p>0,05	p<0,05*	p>0,05
Endometahs.	3,96	1,91		p>0,05	p>0,05
Tubliseal	13,76	8,68	0,65		p>0,05
AH 26	6,95	2,77	0,73	2,91	
MRS - AGAR					
Materijal	Ketac-Endo	Apexit	Endomethasone	Tubliseal	AH 26
Ketac-Endo		p>0,05	p>0,05	p>0,05	p>0,05
Apexit	0,70		p>0,05	p<0,05*	p>0,05
Endometahs.	0,71	1,91		p>0,05	p>0,05
Tubliseal	0,46	8,69	0,65		p>0,05
AH 26	3,71	2,77	0,73	2,91	

Tabela 4 Statistička značajnost razlika u *z.inhibicije rasta* na *S.mutans* među testiranim materijalimaTable 4. Statistical significance among tested sealers in regard to growth inhibition zone of *S. mutans*

KRVNI AGAR					
Materijal	Ketac-Endo	Apexit	Endomethasone	Tubliseal	AH 26
Ketac-Endo		-	p>0,05	p>0,05	p<0,001**
Apexit	-		-	-	-
Endometahs.	2,35	-		p>0,05	p<0,001**
Tubliseal	2,40	-	0,63		p<0,001**
AH 26	13,59**	-	7,79**	9,79**	
TYC-SB - AGAR					
Materijal	Ketac-Endo	Apexit	Endomethasone	Tubliseal	AH 26
Ketac-Endo		-	p>0,05	p>0,05	p<0,01**
Apexit	-		-	-	-
Endometahs.	2,67	-		p>0,05	p<0,01**
Tubliseal	5,29*	-	1,51		p<0,001***
AH 26	16,94**	-	13,40**	33,83***	

It has been statistically confirmed that freshly mixed AH26 has the strongest antimicrobial activity against *S. mutans* compared to other tested materials. (Table 4.)

In this microbiological study, the activity of freshly mixed materials against *L. casei* on two types of agar (MRS and blood) was evaluated. (Table 5)

The greatest average value for growth inhibition zone of *L. casei*, i.e. the strongest antimicrobial effect was recorded for AH26, especially on MRS agar (37.3 mm) as well as on blood agar (18.3 mm). This was followed by eugenol based sealers, Tubliseal and Endomethasone on blood agar (22.7 mm and 20 mm, respectively). The lower antibacterial effect of these two sealers was found against *L. casei* on MRS agar (11.7 mm and 10.7 mm, respectively). These were followed by Apexit with similar activity against *L. casei* on both types of agar (15 mm on blood and 16.7 mm on MRS agar). Ketac Endo showed similar and lower activity than previous against *L. casei* on both types of agar. (Table 5)

Na osnovu dobijenih rezultata utvrđena je i statistička značajnost razlika prosečnih vrednosti prečnika z.inhibicije rasta *L.casei* za pojedine materijale, (Tabela 6).

Ketac Endo ima statistički značajno manji antimikrobni efekat u poredjenju sa AH 26, Apexit-om i Tubliseal-om na *L.casei* na krvnom agaru. Na MRS-u nisu potvrđene statistički značajne razlike medju ispitivanim materijalima Tabela 6.

Diskusija

ADT test je metoda koja se najčešće koristi za procenu antimikrobnog dejstva dentalnih materijala, (11,12). Na rezultate dobijene ovom metodom utiče kontakt materijala sa agarom, mogućnost difuzije materijala u agar (što zavisi od vremena vezivanja), viskoznosti agara, inkubacije, temperature itd. Najveći nedostatak ove metode jeste što ne može da diferencira baktericidno od bakteriostatičkog dejstva materijala. Na rezultate testa utiče ne samo toksičnost materijala, već i mogućnost rastvaranja materijala u vodenom miljeu agara, kao i mogućnosti difuzije koja zavisi kako od rastvorljivosti materijala tako i od vremena stvrdnjavanja. Materijal koji lakše difunduje može imati veći prečnik zone inhibicije rasta mikroorganizama. Brojni autori su se ipak složili da ova metoda omogućava poređenje više vrsta materijala i ukazuje koji od njih ima veći antimikrobni efekat u kanalu korena, (5,12).

Izuzetno jak antibakterijski potencijal imao je sveže pripremljen AH 26, na oba test mikroorganizma. Materijal se znatno duže vezuje (oko 36 sati), pa je i mogućnost difuzije u agar veća što može da ima za posledicu i širu zonu inhibicije rasta,(12).U vezi s tim su interesantna zapažanja Spangberga i sar, koji ovu osobinu materijala pripisuje oslobađanju formaldehida iz heksametilenteetraamina u količini koja se povećava dva dana po vezivanju. Koncentracija formaldehida je po vezivanju materijala približno 200 puta veća nego kod sveže zamešanog materijala i smanjuje se sledećih 7 dana, 13. Rezultati mogu biti uslovljeni i boljom difuzijom same antimikrobne komponente, formaldehida, koji se oslobađa u procesu polimerizacije ili bisfenol diglicid kod AH Plus kroz agar (7,13,14,15).

Endomethasone i Tubliseal, materijali na bazi cink oksid eugenola imaju izuzetno jak antibakterijski efekat zahvaljujući oslobađanju eugenola i posle vezivanja paste, što potvrđuju i drugi autori,(16,17). Brojna ispitivanja ukazuju na izuzetno dobro antimikrobno dejstvo ovih materijala (18,19,20). Visoke koncentracije ZnO imaju malu ulogu u ovom dejstvu ali najveća aktivnost pripada eugenolu,(21). Rezultati Orstavika i sar. 1988. ukazuju na dobru dezinfekcionu ulogu ZOE materijala (Proco Sol) koji pokazuje dubinu dezinfekcione zone dentinskih tubula inficiranih *E.faecalis*-om i do 250 μm ,(22).

Ketac Endo was found to have significantly lower antibacterial activity against *L. casei* on blood agar than AH26, Apexit and Tubliseal. On MRS agar there were no statistically significant differences among tested materials. (Table 6)

Discussion

ADT is the most commonly used method for evaluating antimicrobial activity of dental materials.(11,12) The results of this method are influenced by the contact between a material and agar, the possibility of material diffusion into agar (depends on the setting time), agar viscosity, incubation, temperature etc. The main drawback of this method is that it cannot differentiate bactericidal from bacteriostatic effect of a material. Test results are influenced not only by material toxicity, but also the possibility of dissolving the material in the water component of agar and the diffusion that depends on material solubility and setting time. Highly diffusible material can produce a large growth inhibition zone. Many authors have agreed that this method can be used to compare materials and show which one has the greater antimicrobial effect in the root canal.(5,12)

Extremely high antibacterial potential was recorded for fresh mixture of AH26 against both tested bacterial species. It takes much longer for the material to set (about 36 hours) so the possibility of its diffusion into agar is greater and so the growth inhibition zone.(12) In regards to this, an interesting remark was made by Spangberg et al, in the sense that formaldehyde is released from hexamethylentetraamine in a quantity that rises two days after setting. Formaldehyde concentration upon material setting is about 200 times higher than in freshly mixed material and drops in the next 7 days. (13) The results of the study can be influenced by better diffusion of antimicrobial component itself, formaldehyde, released during polymerization or bisfenol diglycide (AH Plus) through agar.7, (13-15)

Endomethasone and Tubliseal, zinc oxide eugenol-based materials, have a very strong antibacterial effect due to eugenol release even after setting, which was confirmed by other researchers.(16-20) Zinc oxide has a small part in this activity whereas eugenol plays the dominant role.(21) The study by Ørstavik et al. (1988) suggested a good disinfective role of zinc oxide eugenol based materials (Proco sol) which exhibited disinfection of dental tubules infected with *E. faecalis* up to 250 μm .(22)

Apexit, a calcium hydroxide based material, freshly mixed exhibited no activity against *S. mutans* on both types of agar. It had a moderate antimicrobial activity against *L. casei*, slightly better than Ketac Endo and similar as AH26. Although many studies suggest strong antibacterial activity of these materials, material setting may interrupt the release of calcium and particularly hydroxyl

Apexit kao materijal na bazi kalcijum hidroksida (sveže pripremljen) je bio u potpunosti neaktivan u pogledu antimikrobnog dejstva na *S.mutans* na obe hranljive podloge. Pokazao je umereno antibakterijsko dejstvo na *I.casei*, nešto bolje od Ketac Endo, a slično AH 26. Iako brojna ispitivanja ukazuju da se radi o materijalima koji imaju jaku antibakterijsku aktivnost vezivanje materijala može ograničiti oslobađanje kalcijum hidroksida posebno OH jona koji su odgovorni za antimikrobna dejstva,(22,23,24). Apexit je materijal na bazi polimerne smole i kalcijum hidroksida koji ima malu rastvorljivost hidroksilnih jona u agaru. Moguće je da sveže pripremljen materijal u početku nije pokazao antibakterijsko svojstvo zbog polimerizacije i kasnijeg odloženog oslobađanja OH jona. To je verovatno razlog slabe antimikrobne aktivnosti. Ovo je u saglasnosti sa nalazima Orstavika i sar., 1988. koji ne nalaze dezinfekcionu ulogu Sealapex-a koji je 4 sata izložen kontaktu sa inficiranim dentinskim tubulima, verovatno zbog polimerizacije,(10,22).

Podaci iz literature takodje potvrđuju slab antibakterijski efekat materijala na bazi kalcijum hidroksida objašnjavajući to slabom rastvorljivošću kalcijum hidroksida i neutralnim pH. Ovo može biti razlog kasnijeg oslobađanja OH jona što uslovljava odloženo antimikrobno dejstvo,(4,6,22).

Antimikrobni efekat kalcijum hidroksidnih materijala zavisi od oslobađanja hidroksilnih jona koji utiču na povećanje pH, (23,24,25). Time se stvara alkalna sredina nepovoljna za rast i razmnožavanje mikroorganizama. Uticaj pH sredine, $pH > 9$ može reverzibilno ili ireverzibilno inaktivirati ćelijsku membranu enzima ili mikroorganizama, čime se gubi biološka aktivnost citoplazmatične membrane. Može dovesti do destrukcije fosfolipida ili nezasićenih masnih kiselina što rezultira gubitkom integriteta citoplazmatične membrane mikroorganizama,(4,6). Međutim Siqueira i sar. ukazuju da kalcijum hidroksidni materijali zbog male rastvorljivosti redukuju oslobađanje hidroksilnih jona čime redukuje alkalnost, koja je osnovni uslov antimikrobnog dejstva,(12). Smatra se da kalcijum iz paste reaguje sa ugljen dioksidom iz tkiva i sprečava respiraciju anaerobnih bakterija. Ispitivanja Cobankara i sar. su potvrdila je da se kalcijum kod Apexit-a i Sealapex-a oslobađa najvećim delom 30-tog dana,(4).

Glas jonomer cement, Ketac Endo je dao umeren i ujednačen antimikrobni efekat na oba testirana mikroorganizma ispitivana u četiri hranljive podloge. Pokazao je manje antibakterijsko svojstvo u odnosu na materijale na bazi epoksi smola i eugenola, a bolje u odnosu na sredstva na bazi kalcijum hidroksida. Treba imati u vidu da se Ketac Endo brzo vezuje (7 min.), što je moglo uticati na manje inhibitorno svojstvo, jer stvrdnjavanjem materijala mogućnost difuzije u agar je manja. Po nalazima Fuss-a i sar., Ketac Endo ima jače antibakterijsko dejstvo 1 sat po pripremi nego kasnije, za razliku od cink oksid eugenolnih materijala koji imaju produženo antimikrobno dejstvo,(26). Shalhav i sar. nalaze jako i kratkotrajno antimikrobno dejstvo glas jonomer cementa Ketac Endo, (8). Ispitivanja Cobankara i sar, slično našim nalazima, utvrđuju slabije antibakterijsko dejstvo u odnosu na paste na bazi cink oksid eugenola, smola i kalci-

ions, which are responsible for antimicrobial effect.(22-24) Apexit is based on resin and calcium hydroxide and possesses low solubility of hydroxyl ions in agar. It is possible that freshly prepared material showed no antibacterial activity due to polymerization and delayed release of hydroxyl ions. This result is in accordance with the study of Ørstavik et al. who reported no antimicrobial activity of Sealapex even after 4 hours of exposure to infected dentin, probably due to polymerization. (10,22)

Literature data confirm low antimicrobial activity of calcium hydroxide based materials explaining it with low solubility of $Ca(OH)_2$ and a neutral pH. This could be the reason for a delayed release of OH^- ions and subsequent antimicrobial activity.(4,6,22)

Antimicrobial effect of calcium hydroxide based materials depends on the release of hydroxyl ions that increase pH.(23-25) Therefore, an alkaline environment is created that is unfavorable for microbial growth. Environmental pH, $pH > 9$, can reversibly or irreversibly inactivate the cell membrane of enzymes or microorganisms which to the loss of biological activity of cytoplasmic membrane. It could also lead to the destruction of phospholipids or unsaturated fatty acids, resulting in the loss of microbial cytoplasmic membrane integrity. (4,6) However, Siqueira et al. have reported that calcium hydroxide based sealers exhibit reduced release of hydroxyl ions due to low solubility, thus reducing alkalinity which is the main precondition for antimicrobial activity.(12) It is believed that calcium from the paste interacts with carbon dioxide from the tissue and disables the respiration of anaerobic bacteria. Another study has confirmed that the highest release of calcium from Apexit and Sealapex occurs on the 30th day.(4)

Glass ionomer based sealer, Ketac Endo, exhibited moderate and uniform antimicrobial effect against both tested microorganisms on four types of agar. Its antibacterial activity was lower than resin based and eugenol based sealers and higher than calcium hydroxide based sealers. One should bear in mind that Ketac Endo sets quickly (7 minutes) which could influence lower inhibitory effect. During setting, the possibility of material diffusion into agar decreases. According to Fuss et al, Ketac Endo possesses stronger antimicrobial potential 1 hour after setting than later, which opposite to zinc oxide eugenol based materials that have a prolonged antimicrobial activity.26 Shalhav et al reported strong and short lasting antimicrobial activity of glass ionomer based material, Ketac Endo.8 The study of Cobankara et al, like ours, found lower antibacterial effect of this material compared to zinc oxide eugenol, resin and calcium hydroxide based sealers.(4) It is believed that fluoride from glass ionomer cement possesses antimicrobial effect, because its release results in the reduction of acid environment produced by bacteria. Polyacrylic acid is also responsible for the antimicrobial effect of these materials.(10) Heling and Chandler reported that antibacterial effect of Ketac Endo against *E. faeca-*

jum hidroksida,(4). Smatra se da fluor iz GJC-a ima antimikrobni efekat, jer se njegovim oslobađanjem redukuje kisela sredina koju stvaraju bakterije. Poliakrilna kiselina je takođe je odgovorana za antimikrobni efekat ovih materijala,10. Heling, Chandler ukazuju da je antibakterijski efekat Ketac Endo na *E.faecalis* povećava posle 7 dana,slično nalazima Coconbara i sar. i da je uslovljeno oslobađanjem jona fluora, (22). Pojedini autori smatraju da je moguće da je antimikrobna komponenta manje rastvorljiva u agaru u odnosu na antimikrobne komponente drugih silera, (4).

Nalazi bakterioloških komparativnih ispitivanja su verovatno posledica antimikrobnih potencijala navedenih materijala koji zavise od njihovog sastava, brzine stvrdnjavanja i sposobnosti difuzije u hranljivi agar. Isto tako pripremljen materijal ima različit potencijal inihibitorne zone počev od 0 mm (Apexit na *S.mutans*) do 26 mm (AH 26).

Zaključak

Na osnovu dobijenih rezultata može se zaključiti da svi testirani materijali za kanalno punjenje pokazuju antibakterijski efekat na referentne sojeve *S.mutans* i *L.casei*, izuzev Apexit-a koji u sveže zamešanom stanju nema antibakterijsko dejstvo na *S.mutans*. Najjači efekat daju materijali na bazi veštačke smole(AH 26) zatim paste na bazi cink oksid eugenola, Tubliseal i Endomethasone.

GJC, Ketac Endo ima umereno i ujednačeno antibakterijsko dejstvo na oba mikroorganizma i sve četiri podloge, što se za razliku od ostalih materijala može pripisati brzom stvrdnjavanju materijala.

lis rose 7 days after material setting, similarly to the report of Cobankara et al, and that this effect was influenced by fluoride ion release.(22) Some researcher think it is possible that the antimicrobial component is less soluble in agar than antimicrobial components of other sealers.(4) The results of comparative bacteriological studies are probably due to antimicrobial potentials of tested materials which depend on composition, setting time and diffusibility into agar. Prepared materials possess different potentials of growth inhibition zone, starting from 0 mm (Apexit against *S. mutans*) up to 26 mm (AH26).

Conclusion

Based on the results of the current study, it can be concluded that all tested sealers possess antibacterial effect against *S. mutans* and *L. casei*, except Apexit which does not exhibit antibacterial effect against *S. mutans* when freshly mixed. The strongest effect was recorded for resin based sealers (AH26) followed by zinc oxide eugenol based pastes, Tubliseal and Endomethasone. Glass ionomer based sealer, Ketac Endo, has a moderate and uniform effect against both tested microorganisms on all four types of agar. Unlike other sealers, this can be attributed to quick material setting.

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