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Suzana Ferk[1], Paris Simeon[2], Jurica Matijević[2], Goranka Prpić Mehičić[2], Ivica Anić[2], Silvana Jukić Krmek[2]

Antibakterijski učinak agregata mineralnog trioksida i amalgama

Antibacterial Effect of Mineral Trioxide Aggregate and Amalgam

[1] Privatna ordinacija u Zagrebu
Private Dental Practice, Zagreb
[2] Zavod za endodonciju i restaurativnu stomatologiju Stomatološkog fakulteta Sveučilišta u Zagrebu
Department of Endodontics and Restorative Dentistry, School of Dental Medicine, University of Zagreb

Sažetak

Svrha: U ovom se istraživanju ispitivao antibakterijski učinak dvaju materijala za retrogradno brtvljenje korijenskog kanala testom bakterijske difuzije agara. **Materijali i postupci:** Ploče krvnog agara inokulirane su zasebno bakterijama *Streptococcus mutans, Streptotoccus mitis, Lactobacillus acidophilus, Staphylococcus aureus* i *Enterococcus faecalis* te polimikrobnom suspenzijom od svih testiranih bakterija. Na inokulirane ploče bili su raspoređeni uzorci materijala i nakon 48 sati inkubacije očitane su zone inhibicije (ZI) u milimetrima promjera. **Rezultati:** Najsnažniji antibakterijski učinak MTA je pokazao kod bakterije *Streptococcus mutans* (ZI =7,6 mm, s.d. 1,0), zatim prema *Streptotoccus mitis* i *Lactobacillus acidophilus* (ZI=7,3 mm, s.d. 0,5). ZI za *Enterococcus faecalis* iznosio je 5,6 milmetara, s.d. 0,7, a za polimikrobnu suspenziju 5,3 milimetra, s.d. 1,0. Jedina bakterija na koju MTA nije imao antibakterijski učinak jest *Staphylococcus aureus*. Amalgam nije pokazao antibakterijski učinak na ni jednu ispitivanu bakteriju. **Zaključak:** MTA je pokazao antibakterijski učinak prema četiri od pet ispitivanih bakterija i polimikrobnih suspenzija, a amalgam nije inhibirao rast ni jedne testirane bakterije.

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Adresa za dopisivanje
Silvana Jukić Krmek
Sveučilište u Zagrebu
Stomatološki fakultet
Zavod za endodonciju i restaurativnu stomatologiju
Gundulićeva, 10000 Zagreb
Tel: +385 1 4802 126
jukic@sfzg.hr

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Uvod

Apikotomija je kirurški zahvat kojim se nastoji sačuvati zub kao funkcionalna i/ili estetska jedinica u slučaju neuspjeha nekirurškog endodontskog liječenja ili nemogućnosti njegova izvođenja (1). S obzirom na to da su neki neuspjesi u endodonciji rezultat neadekvatnog čišćenja i brtvljenja korijenskog kanala zuba, nužno je vršak reseciranog zuba retrogradno puniti (2) kako bi se spriječila komunikacija i izlaz antigena iz korijenskog kanala u periapeksno tkivo. Idealan materijal za retrogradno punjenje trebao bi potpuno brtviti apikalni dio korijena, biti netoksičan, ne resorbirati se, biti dimenzijski stabilan te jednostavan za rukovanje, radioopakan i biokompatibilan (2,3). Također je poželjno da ima baktericidan učinak ili barem bakteriostatski.

Premda su danas na tržištu dostupni mnogi proizvodi za retrogradno punjenje, dosad ni jedan nije zadovoljio sve uvjete prijeko potrebne za idealan materijal. Do nedavno se dentalni amalgam najčešće rabio za retrogradne kavitete (1). Taj je materijal dobro ispitan i često se u istraživanjima rabi kao standard (4). Postoje razne vrste amalgama za retrogradne kavitete. Razlikuju se u osjetljivosti na koroziju, uglavnom zbog razlika u sastavu, posebice u sadržaju bakra i cinka. Amalgam se može kombinirati s lakom koji se postavlja na stijenke ka-

Introduction

Apicoectomy is a surgical procedure which tends to preserve the tooth as a functional and/or aesthetic unit in the case of unsuccessful non-surgical endodontic treatment, or when such treatment is not possible (1). Given that some failures in endodontics result from inadequate root canal cleaning and obturation, it is essential to retrogradly obturate the end of the resected root (2) to prevent communication and egress of antigen from the root canal into the periapical tissue. The ideal retrograde root filling material should completely seal the apical part of the root, be non-toxic, non-resorbable, dimensionally stable, easy to handle, radio opaque and biocompatible (2, 3). It should also have a bactericidal or at least a bacteriostatic effect.

Nowadays, many retrograde filling materials are commercially available, but so far none have fulfilled all the necessary requirements for an ideal material. Until recently, dental amalgam was the most common material used for retrograde cavities (1). This material was studied thoroughly and often used in research as a standard material (4). There are various types of amalgam for retrograde fillings. They differ in their sensitivity to corrosion, mainly due to differences in composition, particularly in copper and zinc content. Amalgam can viteta prije retrogradnog punjenja. Amalgam se na njih ne veže, ali se može lako postaviti i kondenzirati posredstvom minijaturnih nosača i nabijača (5). Nedostatci amalgama su inicijalno curenje pokraj ispuna što je rezultat početnog skvrčavanja, stvaranje korozivnih nusproizvoda, mogućnost kontaminacije živom i kositrom, osjetljivost na vlagu, potreba za retentivnim oblikom kaviteta, moguće obojenje tkiva te to što se raspršeni komadići materijala teško uklanjaju i ne mogu se resorbirati (1).

Posljednjih godina razvijen je novi materijal Mineral Trioxide Aggregate (MTA). To je hidrofilni endodontski cement izrazito biokompatibilan i sposoban stimulirati cijeljenje i osteogenezu. Proizvodi se kao prah koji se sastoji od finih čestica trioksida (trikalcijev oksid, silikatni oksid i bizmutov oksid) te ostalih hidrofilnih čestica (trikalcijev silikat i trikalcijev aluminat) odgovornih za fizikalna i kemijska svojstva ovog agregata. Stvrdnjava se u prisutnosti vode te nastaje koloidni gel čiji pH iznosi 12,5 (6). Stvrdnjavanje gela traje tri do četiri sata. Taj se cement razlikuje od ostalih materijala trenutačno dostupnih na tržištu prema biokompatibilnosti, antibakterijskom učinku, marginalnoj adaptaciji i izvrsnom brtvljenju te hidrofilnosti (7).

Biokompatibilnost tog cementa očituje se u odsutnosti citotoksičnosti kada MTA dođe u doticaj s fibroblastima i osteoblastima te stvaranju dentinskog mosta ako se rabi kao sredstvo za prekrivanje pulpe (8,9). Rast cementa, parodontnog ligamenta i kosti pokraj MTA-a, kada se on rabio za zatvaranje perforacija korijena (10) ili kao retrogradno punjenje u apikalnoj kirurgiji (11), potvrđen je u mnogim istraživanjima.

U dosadašnjim su istraživanjima Torabinejad i suradnici (12,13,14) pronašli da MTA statistički znatno smanjuje mikropropuštanje boje i bakterija te da bolje rubno prianja za zubno tkivo, a i biokompatibilnost mu je veća nego kod amalgama Super-EBA i IRM. To posljednje očituje u stvaranju novog cementa koji se odlaže na reseciranom vršku korijena prekrivajući retrogradni ispun. Upravo je to ono što izdvaja MTA i stavlja ga iznad svih materijala dostupnih na tržištu.

Svrha ovog istraživanja bila je testom difuzije agarom proučiti antibakterijsko djelovanje amalgama i MTA na fakultativno anaerobne bakterije dominantne u periapikalnom procesu.

Materijali i postupci

U radu je ispitivan antibakterijski učinak amalgama bez cinka (Amalcap, Vivadent, Schaan, Liechteinstain) i Mineral Trioxide Aggregata (ProRoot, Dentsplay/Maileffer, Konstanz, Njemačka). Antibakterijski učinak analiziran je pomoću pet fakultativno anaerobnih bakterija, pojedinačno i pomiješanih tako da tvore polimikrobnu suspenziju koja najvjernije simulira situaciju *in vivo* te su testirani uzorci materijala i na svaki bakterijski soj zasebno. Bakterije odabrane za ispitivanje bile su *Streptococcus mutans* 6715 WT, *Streptotoccus mitis* ATCC 6249*, Lactobacillus acidophilu* NCTC 1723, *Staphylococcus aureus* ATCC 29213 i *Enterococcus faecalis* ATCC 29212.

be used in combination with a varnish that is placed on the cavity walls before the retrograde filling. Amalgam does not bond to the cavity walls, but can easily be placed and condensed with miniature carriers and pluggers (5). Disadvantages of amalgam are: initial marginal leakage as a result of initial shrinkage, formation of corrosive by-products, possible mercury and tin contamination, sensitivity to moisture, need for a retentive cavity shape, and possibility of tissue discoloration and scattering of amalgam particles which are difficult to remove and cannot be resorbed (1).

In recent years, a new material, Mineral Trioxide Aggregate (MTA), was developed. MTA is a hydrophilic endodontic cement, extremely biocompatible and stimulates healing and osteogenesis. It is a powder that consists of fine particles of trioxide (tricalcium oxide, silicate oxide and bismuth oxide) and other hydrophilic particles (tricalcium silicate and tricalcium aluminate) responsible for the physical and chemical properties of this aggregate. It solidifies in the presence of water, creating a colloidal gel whose pH is 12.5 (6). The gel solidifies in between three and four hours. This cement differs from other materials currently present on the market because of its biocompatibility, antibacterial effect, marginal adaptation, excellent sealing properties and hydrophilic nature (7).

Biocompatibility of this cement is demonstrated by the absence of cytotoxicity when MTA comes into contact with fibroblasts and osteoblasts, and by the formation of dentin bridges when it is used as a pulp capping material (8, 9). The growth of cement, the periodontal ligament and bone adjacent to MTA when used to repair root perforations (10) or as a retrograde filling material in apical surgery (11) has been confirmed in numerous studies.

In previous studies, Torebinajad et al. (12, 13, 14) found that MTA significantly reduced microleakage of dye and bacteria, had better marginal adhesion properties towards dental tissues and better biocompatibility than amalgam, Super-EBA and IRM. The latter feature is demonstrated by the formation of new cement on the resected root, covering the retrograde root filling. The formation of cement is exactly what sets MTA apart from other materials present on the market today.

The purpose of this study was to investigate the antibacterial properties of MTA and amalgam against facultative anaerobic bacteria dominant in periapical lesions using the agar diffusion test.

Materials and methods

This study examined the antibacterial effect of zinc-free amalgam (Amalcap, Vivadent, Schaan, Liechteinstain) and Mineral Trioxide Aggregate (ProRoot, Dentsply/Maileffer, Konstanz, Germany). Sample materials were tested using five facultative anaerobic bacteria, individually and combined to form a polymicrobial suspension that most closely simulates the *in vivo* situation. Sample materials were also tested on each bacterial strain separately. The bacteria used in this study were *Streptococcus mutans* 6715 WT, *Streptococcus mitis* ATCC 6249, *Lactobacillus acidophilus* NCTC 1723, *Staphylococcus aureus* ATCC 29213, and *Enterococcus faecalis* ATCC 29212.

Polipropilenske tubice Ependorf volumena 1,5 ml (Sigma, Aldrich, SAD) prerezane su i od njih su oblikovani standardizirani prstenovi promjera tri milimetra koji su poslužili kao kalupi za ispitivane materijale. Prstenovi su sterilizirani 30 minuta u autoklavu na temperaturi od 120°C i tlaku od 300 kP.

Ispitivani materijali zamiješani su prema naputku proizvođača i stavljeni u prstenove.

Sterilni prstenovi uronjeni u tekućinu eugenola ZnOE cementa služili su kao pozitivna kontrola, a oni sterilni postavljeni na ploče krvnog agara inokulirani kontaminiranim bujonom bili su negativna kontrola.

Ispitivane bakterije, prije toga kultivirane na pločama krvnog agara, inokulirane su u Shedlerov bujon i ostavljene preko noći u termostatu na 37º C. Na dan eksperimenta obavljeno je razrjeđenje bakterija od $9x10^8$ CFU/ml (Mc Farlandov standard) u svježem mediju (otopina barij sulfata, bio Merieux sa, 69280 Marcy l'Etoile, Francuska). Čistoća kulture potvrđena je uniformnošću kolonija na pločama nakon razrjeđenja, mobilnosti te morfoloških i gram karakteristika bakterija.

Na ploče krvnog agara nasađeno je 1/10 mililitara svake bakterijske kulture koje su ravnomjerno raspoređene preko cijele površine ploče sa sterilnom ezom. Isto je učinjeno i s polimikrobnom suspenzijom. Pripremljeni uzorci ispitivanih materijala iz kalupa postavljeni su jedan do drugoga na površine ploča krvnog agara s razmakom od najmanje dva centimetra. Ploče su inkubirane 48 sati na 37º C u bubnju s anaerobnim uvjetima – Jar s Gas-Pakom (BBL, Anaerobic System Envelopes, Becton Dickinson and Company, Cockeysvill, MD 21030 SAD). Testirano je 12 replika za svaki ispitivani materijal.

Rezultati antibakterijskog učinka izraženi u milimetrima promjera zone inhibicije rasta bakterija i statistički su obrađeni. Razlike između ispitivanih bakterija testirane su One and Two-Way analizom varijance (Anova) i Student T-testom ($p<0,05$), a Schefféov post hoc test koristio se za analizu razlika između pojedinih bakterija.

Rezultati

Parametri deskriptivne statistike antibakterijskog učinka materijala za retrogrdno brtvljenje prikazani su u Tablici 1. Kod svih pet ispitanih vrsta bakterija nije pronađen antibakterijski učinak amalgama, a MTA je pokazao antibakterijski

Eppendorf polypropylene tubes with a volume of 1.5 ml (Sigma, Aldrich, USA) were cut to from standardized rings 3 mm in diameter, which were used as molds for the tested material. The rings were sterilized in an autoclave at a temperature of 120 ° C and a pressure of 300 kPa for 30 minutes.

The materials were prepared according to the manufacturer's instructions and placed into the rings. The sterile rings were immersed in a liquid eugenol, from ZnOE cement, and served as a positive control while sterile rings placed on blood agar plates inoculated with contaminated broth served as a negative control.

The tested bacteria, previously cultivated on blood agar plates, were inoculated in a Stedler broth and left overnight in a thermostat at 37 º C. On the day of the experiment, bacteria were diluted to $9x 10^{8}$ CFU / ml (Mc Farland Standard no. 3) into a fresh medium (Barium sulphate suspension, bioMerieux SA, 69,280 Marcy l'Etoile, France). The evidence of culture purity was given by uniformity of colony form on a dilution streak plate, motility, morphological and gram characteristics.

Blood agar plates were inoculated with 1/10 ml of each bacteria culture, evenly distributed across the entire plate surface with a sterile swab. The same was done with the polymicrobial suspension consisting of equal parts of each bacterial species. The prepared samples of test material removed from the molds were placed on the blood agar plates at a distance of at least 2 cm apart. The plates were incubated at 37 º C for 48 hours in anaerobic conditions (Jar with Gas-Pak Protection BBL, Anaerobic System Envelopes, Becton Dickinson and Company, Cockeysvill, MD 21030 USA). Twelve replicas were tested for each tested material.

The results of antibacterial effect were expressed in millimeters as the diameter of the bacterial growth inhibition zone and were statistically analyzed. The differences among materials were tested using One and Two-Way Analysis of Variance (ANOVA), Student T-test ($p <0.05$) and Scheff's post hoc test for differences among bacteria in one material.

Results

Descriptive statistical parameters of antibacterial effects of root-end filling materials are shown in Table 1. Amalgam did not show antibacterial effects against any of the 5 bacterial species tested, while MTA exhibited activity. The antibac-

Tablica 1. Deskriptivni parametri antibakterijskog učinka materijala za retrogradno brtvljenje
Table 1 Descriptive parameters of antibacterial effect of root-end filling material

	N	MTA		Amalgam	
		M	sd	M	sd
Streptococcus mitis	12	7.3	0.62	0.0	0.00
Streptococcus mutans	12	7.6	1.00	0.0	0.00
Lactobacillus acidophilus	12	7.3	0.49	0.0	0.00
Enterococcus faecalis	12	5.6	0.67	0.0	0.00
Staphylococcus aureus	12	0.0	0.00	0.0	0.00
Polimikrobna suspenzija • Polymicrobial suspension	12	5.3	1.07	0.0	0.00

Legenda • Legend:
M – aritmetička sredina promjera zone inhibicije u milimetrima • arithmetic mean of diameter of the inhibition zone in millimetres
s.d. – standardna devijacija • standard deviation
N – broj mjerenja • number of measurements

učinak. Analizom varijance uspoređen je antibakterijski učinak materijala za retrogradno brtvljenje, odnosno MTA-a i amalgama bez cinka kod pet od šest testiranih vrsta bakterija. Kod bakterije *S. aureus* nije se obavljala statistička analiza i to zato što je promjer zone inhibicije i kod MTA i kod amalgama u svim mjerenjima bio jednak nuli, tj. kod te bakterije ni MTA ni amalgam nisu pokazali nikakav antibakterijski učinak. MTA se statistički značajno razlikuje od (nultog) učinka amalgama. Antibakterijski učinak MTA i amalgama kod svih se pet analiziranih vrsta bakterija statistički značajno razlikuje ($p<0,05$). MTA je pokazao statistički veći antibakterijski učinak na S*treptococcus mitis*, S*treptococcus mutans* i *Lactobacilus acidophilus* (df=11, $p<0,05$) u odnosu prema *Enterococcus faecalis* i polimikrobnu suzpenziju.

Rasprava

Od materijala za brtvljenje retrogradnih kaviteta ispitivanih u ovom radu, amalgam bez cinka uopće nije pokazao antibakterijski učinak ni kod jednog testiranog soja bakterija, ni kod polimikrobnog markera. Taj nalaz u skladu je s radom Torabinajeda i suradnika (15) jer ni u njihovu istraživanju amalgam nije pokazao antibakterijski učinak, a kod ostalih ispitivanih materijala djelomice je bio prisutan. Istraživanja Glassmana i njegovih kolega (16), zatim Orstavika (17), Tobiasa i suradnika (18) te Morriera i njegova tima (19) u suprotnosti su s tim rezultatima. U tim istraživanjima procijenjen je antibakterijski učinak različitih vrata amalgama na fakultativne bakterije. Rezultati su pokazali da većina legura amalgama pokazuje barem neki antibakterijski učinak. Razlika u rezultatima može se pripisati dostupnim nutrijentima, razini parcijalnog pritiska kisika, razdoblju inkubacije, metodi procjene i različitoj laboratorijskoj metodologiji. Osim poznatih prednosti testa difuzije agarom, korištenog u ovom istraživanju, postoje i ograničenja kao što su različita topljivost ispitivanih materijala, rizik od termičkoga šoka tijekom pripreme pokrivača agara, ograničeno vrijeme ekspozicije te moguća apsorpcija vode iz agara (20).

Kod svih pet ispitanih vrsta bakterija i polimikrobne suspenzije, amalgam uopće nema antibakterijski učinak, a MTA ga ima kod bakterija *Streptococcus mitis, Streptococcus mutans, Lactobacillus acidophilus, Enterococcus faecalis* i polimikrobne suspenzije, što je u skladu s rezultatima Torebinejada i suradnika (15). Za bakteriju *Staphylococcus aureus* nije bila obavljena statistička analiza, jer je promjer zone inhibicije i kod MTA i kod amalgama u svim mjerenjima bio jednak nuli. Antibakterijski učinak MTA može biti posljedica njegova visokog pH (31) ili otpuštanja lako difundiranih supstancija po inokuliranim pločama hranjivog medija. Jedna od njih može biti arsen, koji se može, premda u vrlo malim količinama, otpustiti iz MTA i cementa Portland u svojem najtoksičnijem, trovalentnom obliku (21). Premda se prvotno smatralo da su glavni sastojci MTA kalcij i fosfat, Camilleri sa suradnicima (22) dokazao je da se materijal primarno sastoji od trikalcijeva i dikalcijeva fosfata koji, kada se hidratiziraju, stvaraju silikatni gel i kalcijev hidroksid, a ne kalcijev fosfat kao što je tvrdio Torebinejad (23). Antibakterijski učinak kalcijeva hidroksida dobro je poznat i analiziran u mnogim istraživanjima (24).

terial effect of MTA and zinc-free amalgam in 4 out of 5 bacterial strains and polymicrobial suspension were compared using analysis of variance. Statistical analysis for *S. aureus* was not carried out because the diameter of the inhibition zone of MTA and amalgam in all the measurements was zero, i.e., neither MTA nor amalgam showed any antibacterial activity against these bacteria. MTA and amalgam showed a statistically significant difference in antibacterial activity against the other tested bacterial strains ($p<0, 05$). MTA showed a statistically significant antibacterial effect on *Streptococcus mitis, Streptococcus mutans* and *Lactobacillus acidophilus* in comparison with *Enterococcus faecalis* and the polymicrobial suspension (df = 11, $p <0.05$).

Discussion

In this study, zinc-free amalgam did not show any antibacterial effects against the tested bacterial strains or the polymicrobial suspension. This finding is in accordance with the work of Torabinajed et al. (15) where amalgam showed no antibacterial activity, while other tested materials showed partial activity. Unlike those results, the results of Glassman et al. (16), Orstavik et al. (17), Tobias et al. (18), and Morrier et al. (19) contradict with the results of this study. In those studies, the antibacterial effects of different types of amalgam were evaluated against various bacteria. Their results revealed that most amalgam alloys showed some antibacterial activity. The difference in results can be attributed to the available nutrients, the level of partial pressure of oxygen, the incubation period, the method of evaluation and different laboratory methodology. Apart from the known advantages of agar diffusion test, which was used in this study, there are some limitations such as different solubility of toxicant in agar, risk of thermal shock when preparing agar overlay, limited exposure time and risk of absorbing water from agar (20).

Amalgam did not show antibacterial activity against all five bacteria tested and polymicrobial suspension. MTA showed antibacterial activity against *Streptococcus mitis, Streptococcus mutans, Lactobacillus acidophilus, Enterococcus faecalis* and a polymicrobial suspension, which is consistent with the findings of Torabinejad et al. (15). Statistical analysis was not carried out for *Staphylococcus aureus*, because the diameter of the inhibition zones for MTA and amalgam in all performed measurements was zero. The antibacterial effect of MTA may be due to its high pH (15) or to the release of easily diffusible substances through the nutrient media of the inoculated plates. One of these substances may be arsenic which can, although in very small amounts, be released from MTA and Portland cement in its trivalent, most toxic form (21). Although, initially it was considered that the main ingredient of MTA was calcium and phosphate, Camilleri et al. (22) proved that the material consists primarily of tricalcium and dicalcium phosphates which when hydrated produce a silicate gel and calcium hydroxide, not calcium phosphate, as claimed by Torabinajed (23). The antibacterial effect of calcium hydroxide is well known and researched in many studies (24).

Kada se razmatra antibakterijski učinak materijala za retrogradno punjenje korijenskih kanala, treba imati na umu bakterije koje se najčešće nalaze kod perzistentne ili sekundarne endodontske infekcije, a razlikuju se od onih u primarnoj infekciji. Novije molekularne tehnike otkrile su, osim *Enterococcus feacalis* poznatog od prije kao bakterije koja se najčešće povezuje s neuspješnim endodontskim liječenjem, druge bakterijske vrste kao što su *Pseudoaramiabcter alactolyticus, Propionibacterium propionicum, Dalister pneumosintes* i *Filifactor alocis* u korijenskim kanalima zuba s periapikalnim procesom (25). Kako bi se bolje shvatio utjecaj materijala za retrogradno brtvljenje na cijeljenje periapikalnog tkiva, potrebno je ispitati njihov antibakterijski učinak na te i druge bakterijske vrste česte u sekundarnoj infekciji korijenskog kanala, kao što su *Porphyromonas* i *Bacteroides*.

S obzirom na pokazani antibakterijski učinak, MTA bi trebao imati prednost pred amalgamom pri odabiru za brtvljenje retrogradnih kaviteta, posebice ako se ima na umu njegova superiornost kod retrogradnog brtvljenja (26,27) i koronarnog mikropropuštanja (27) te osteogenog potencijala koji posjeduje (21). Istraživanja Torebinajeda i suradnika (12,13), Hollanda i njegovih kolega (29) te Yaltirika i suradnika (30) dokazala su rast cementa, periodontnog ligamenta i rast kosti u blizini MTA, kada se rabio za brtvljenje korijenskih perforacija te pri brtvljenju retogradnih kaviteta. Studija Torebinajeda i suradnika (12) pokazala je da je MTA jedini materijal na koji vlaga i/ili kontakt s krvlju nemaju negativan učinak tj. ne utječu na njegovu sposobnost brtvljenja. Taj podatak, uzevši u obzir da je tijekom kirurškog zahvata apikotomije krv neizbježna, dodatno pridonosi izboru MTA za retrogradno brtvljenje korijenskog kanala.

Zaključak

Na kraju možemo reći da je MTA pokazao antibakterijski učinak među materijalima za retrogradno brtvljenje korijenskog kanala kod četiriju ispitanih vrsta bakterija *(Streptococcus mitis, Streptococcus mutans, Lactobacillus acidophilus, Enterococcus faecalis*) i polimikrobne suspenzije. Antibakterijski učinak amalgama i MTA izostao je kod bakterije *Staphylococcus aureus*. Amalgam nije pokazao antibakterijski učinak ni kod jedne ispitivane bakterije zasebno, a ni kod polimikrobne suspenzije.

Considering antibacterial properties of root end filling materials, one should bear in mind the most frequent bacterial strain associated with persistent or secondary intraradicular infection which differ from its primary form. New molecular techniques revealed that beside *Enterococcus feacalis*, which was previously known as most frequent bacteria involved in endodontic failures, there are other species such as *Pseudoaramiabcter alactolyticus, Propionibacterium propionicum, Dalister pneumosintes* and *Filifactor alocis* present in root canals of teeth with periradicular lesion (25). For better understanding of influence of root-end filling material on periapical healing, these species and some other bacterial strains common in secondary root canal infection such as *Porphyromonas* and *Bacteriodes species* should be used in studies of their antibacterial properties. Given the demonstrated antibacterial effect, MTA should be used as a material for filling a retrograde cavity instead of amalgam, especially if one keeps in mind its superiority in sealing ability (26, 27) lower coronary microleakage (28) and the osteogenic potential (21). The research of Torebinejad et al. (12, 13), Holland et al. (29) and Yaltirik et al. (30) showed the growth of cementum, the periodontal ligament and the bone adjacent to MTA when used in sealing root perforations and retrograde cavities. Torebinejad et al. (12) showed that MTA is the only material which moisture and/or contact with blood does not have a negative effect on, i.e., does not affect its sealing ability. Considering the fact that during an *apicoectomy* blood is inevitable, this further contributes to the choice of MTA as a root-end filling material.

Conclusion

It can be concluded that, among root-end filling materials, MTA displayed antimicrobial activity against four tested bacterial species (*Streptococcus mitis, Streptococcus mutans, Lactobacillus acidophilus, Enterococcus faecalis*) and a polymicrobial suspension. Antimicrobial activity of amalgam and MTA was not present against *Staphylococcus aureus*. Amalgam showed no antibacterial effect against any of the tested bacteria, neither separately nor as a polymicrobial suspension.

Abstract

The purpose of this study was to evaluate the antibacterial effect of root-end filling materials, MTA and amalgam, by agar diffusion test. **Materials and methods:** Blood agar plates were inoculated with bacteria *Streptococcus mutans, Streptococcus mitis, Lactobacillus acidophilus, Staphylococcus aureus* and *Enterococcus faecalis* each, and a polymicrobial suspension consisting of all the tested bacteria. Material samples were divided among the inoculated plates, and after 48 hours of incubation, the diameters of the inhibition zone (IZ) were recorded. **Results:** The strongest antibacterial effect of MTA was against *Streptococcus mutans* (IZ = 7.6 mm, sd 1.0), followed by *Streptococcus mitis* and *Lactobacillus acidophilus* (IZ = 7.3 mm, sd 0.5). The inhibition zone (IZ) for *Enterococcus faecalis* was 5.6 mm (sd 0.7), and for the polymicrobial suspension 5.3 mm (sd 1.0). *Staphylococcus aureus* was the only bacteria MTA did not show any antibacterial effect against. Amalgam showed no antibacterial effect against any of the bacteria tested. **Conclusion:** MTA showed antibacterial effect against 4 of 5 tested bacteria and the polymicrobial suspension, while amalgam did not inhibit the growth of any of the bacteria tested.

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Address for correspondence
Silvana Jukić Krmek
University of Zagreb School of Dental Medicine
Department of Endodontics and Restorative Dentistry,
Gundulićeva, 10000 Zagreb
Tel: +385 1 4802 126
jukic@sfzg.hr

Key words
Root Canal Filling Materials; Bacteria, Anaerobic; Anti-bacterial Agents; Dental Amalgam; Streptococcus; Staphylococcus aureus; Enterococcus faecalis; Lactobacillus acidophilus

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