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José C. Provenzano, Julio C. M. Oliveira, Flávio R. F. Alves, Isabela N. Rôças, José F. Siqueira Jr, Milton de Uzeda

Antibakterijska svojstva retrogradnih punila korijenskih kanala

Antibacterial Activity of Root-end Filling Materials

Zavod za endodonciju Stomatološkog fakulteta Sveučilišta Estácio de Sá, Rio de Janeiro, Brazil
Department of Endodontics, Faculty of Dentistry, Estácio de Sá University, Rio de Janeiro, RJ, Brazil.

Sažetak
Svrha istraživanja bila je ocijeniti i usporediti antibakterijsku učinkovitost pet retrogradnih punila korijenskih kanala. **Materijal i metode:** Metodom difuzije u agru ocijenjena je zona inhibicije bakterijskog rasta na podlozi pet retrogradnih punila korijenskih kanala: Pro Root MTA, cementa Portland, Super-EBA, IRM-a, Sealera 26 te amalgama. Za ciljane mikroorganizme bilo je odabrano pet anaeroba (*Porphyromonas gingivalis*, *Parvimonas micra*, *Fusobacterium nucleatum*, *Propionibacterium acnes* i *Eubacterium saburreum*) te dva fakultativna (*Actinomyces viscosus* i *Enterococcus faecalis*). **Rezultati:** Sealer 26 postigao je najveću inhibiciju rasta. Punila IRM i Super-EBA spriječila su rast samo Porphyromonas gingivalis, ali učinak je bio znatno slabiji u usporedbi s djelovanjem Sealera 26. Kao neučinkovita antibakterijska sredstva pokazali su se MTA, cement Portland i amalgam. **Zaključak:** Rezultati istraživanja potvrdili su da Sealer 26 ima najjače antibakterijsko djelovanje u usporedbi s ostalim često upotrebljavanim apikalnim punilima korijenskih kanala.

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Adresa za dopisivanje
José Claudio Provenzano, DDS, MSc
Rua Lins de Vasconcellos, 195 casa 9
Rio de Janeiro, RJ
Brazil 20710-130
odontojcp@gmail.com

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Uvod

Posljednjih četrdesetak godina u istraživanjima je potvrđeno da su bakterije neobično važne u nastanku apikalnog parodontitisa (1). Zbog toga se danas endodoncija smatra kliničkom disciplinom koja se bavi prevencijom i kontrolom infekcije endodontskog područja koja uzrokuje apikalni parodontitis. Bakterije koje ostanu u kanalu nakon endodontskog tretmana ili one koje na neki drugi način inficiraju napunjeni kanal, smatraju se glavnim uzročnicima apikalnog parodontitisa nakon tretmana (2). Ekstraradikularne infekcije također se smatraju uzrokom perzistentnih apikalnih parodontitisa (3).

Kirurški zahvat u slučaju periradikularnog parodontitisa indiciran je kod neuspjele revizije ili ako revizija nije moguća. Taj je kirurški zahvat koristan jer omogućuje kiretažom, dakle mehanički, ukloniti periradikularnu infekciju te apikotomijom intraradikularnu infekciju. Ako u kanalu postoje bakterije, punjenjem apikalne trećine kanala brtvi se korijenski kanal pa one preostale ostaju u njemu i ne ugrožavaju ishod operativnog zahvata. Zato se očekuje da materijali za brtvljenje apikalne trećine korijenskog kanala dobro prianjaju i imaju antibakterijska svojstva. (4,5).

Svrha ovog istraživanja bila je usporediti antibakterijski učinak pet materijala za brtvljenje apikalne trećine korijenskog kanala (Pro Root MTA, cementa Portland, Super-EBA, IRM-a, Sealera 26 i amalgama) na pet anaerobnih bakterija i dvije fakultativno anaerobne, koristeći se testom difuzije agra.

Introduction

Over the last four decades, bacteria have largely been demonstrated to play an indisputable role in the causation of apical periodontitis (1). Thus, endodontics has been regarded as the clinical discipline dealing with prevention and control of endodontic infections which are the cause of apical periodontitis. Bacteria persisting in the root canal after treatment or invading the root canal after professional intervention are seen as the major cause of post-treatment apical periodontitis (2). Extraradicular infections can also be the cause of persistent apical periodontitis lesions (3).

Periradicular surgery is indicated in cases of endodontic treatment failure where retreatment has not succeeded or is not feasible at all. Periradicular surgery remains an important therapeutic approach in dealing with treatment failure and thus saving the tooth. This procedure has a great advantage of being able to mechanically remove both the extraradicular infection through curettage of the lesion and the apical intraradicular infection through apicoectomy. If there are bacteria still remaining in the canal, the root-end filling procedure has the opportunity to seal the apical terminus of the canal and entomb these residual bacteria which could otherwise compromise the surgery outcome. Therefore, the root-end filling materials are expected to have, among several other properties, a good sealing ability and antibacterial effectiveness (4,5).

The aim of the present study was to compare the antibacterial effectiveness of five materials used for root-end fillings

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(Pro Root MTA, Portland cement, Super-EBA, IRM, Sealer 26, and amalgam) on five anaerobic bacterial species and two facultative bacterial species, utilizing the agar diffusion test.

Materijali i metode

U istraživanju su se rabili sljedeći materijali za apikalno brtvljenje korijenskih kanala: Pro Root MTA (Dentsply Tulsa Dental, Tulsa, OK, SAD); cement Portland (Cia. de Cimento Itaú, Itaú de Minas, MG, Brazil); Super-EBA (Harry J. Bosworth, Skokie, IL, SAD); IRM (Dentsply Caulk, Milford, DE, SAD); Sealer 26 (Dentsply, Petrópolis, RJ, Brazil) i amalgam (Dispersalloy, Dentsply, Petrópolis, RJ, Brazil). Svi su se pripremali prema uputama proizvođača, osim cementa Portland koji je bio pripremljen slično kao i MTA te Sealer 26 – dakle, imao je nešto gušću konzistenciju nego što je preporučuje proizvođač (omjer; 4:1, v:v, prah:smola).

Bili su odabrani sljedeći bakterijski sojevi: *Porphyromonas gingivalis* (ATCC 33277); *Parvimonas micra* (ATCC 33270); *Fusobacterium nucleatum* subsp. *polymorphum* (ATCC 10953); *Actinomyces viscosus* (own isolate); *Propionibacterium acnes* (own isolate); *Eubacterium saburreum* (ATCC 33271) i *Enterococcus faecalis* (ATCC 29212).

Nakon aktivacije iz originalnih sojeva, liofilizirane ili zamrznute u obranom mlijeku, vrste bakterija čuvane su do uporabe u prikladnom mediju.

Kulture bakterija koje su odležale preko noći koristile su se za inokuliranje. Fakultativni anaerobi uzgajani su na triptikaza-sojinom agaru/hranilištu (TSB) (Difco, Detroit, MI, SAD), a anerobi u prereduciranom anaerobno steriliziranom srčano-moždanom infuzijskom agaru/hranilištu (BHI-PRAS) (Difco) dopunjenom heminom (5 µg/ml) i vitaminom K1 (1 µg/ml). Zamućenost inokuluma BHI-PRAS pripremljenog za anaerobe te inokuluma TSB za ostale mikroorganizme, bila je prilagođena na zamućenost od 0,5 McFarlanda BaSO$_4$ standarda (približno 1,5 x 10^8 CFU[*]).

Petrijeve zdjelice koje sadržavaju agar Brucella (Difco) obogaćen 5-postotnom defibriniranom ovčjom krvlju te dopunjen heminom i vitaminom K1 inokulirane su anaerobnim bakterijama. U one u kojima je bio TSB soj, unesene su fakultativne anaerobne bakterije. Inokulacija je obavljena sterilnim aplikatorima s pamučnim vrhom te je na taj način bila premazana površina agra. Šest krugova dubine pet milimetara i promjera šest milimetara bilo je izbušeno u svakom agru te ispunjeno tek pripremljenim punilima. Nakon toga su Petrijeve zdjelice ostavljene na sobnoj temperaturi približno deset minuta kako bi se inokulum apsorbirao. Svi postupci ponovljeni su tri puta.

Zdjelice agra inokulirane anaerobnim bakterijama pet su dana bile inkubirane u anaerobnim posudicama na temperaturi od 37ºC. Anaerobni uvjeti postignuti su generatorom GasPak Plus (BBL, Becton-Dickinson Microbiology Systems, Cockeysville, MD, SAD).

Zdjelice agra s fakultativnim anaerobima inkubirane su aerobno na 37ºC i to od 24 do 48 sati. Antimikrobni učinak svakog punila bio je određen mjerenjem promjera područja inhibicije izraženim u milimetrima. Kao referentna točka uzet je promjer od šest milimetara.

Materials and Methods

In this study, the following materials used in root-end fillings were tested: Pro Root MTA (Dentsply Tulsa Dental, Tulsa, OK, USA); Portland cement (Cia. de Cimento Itaú, Itaú de Minas, MG, Brazil); Super-EBA (Harry J. Bosworth, Skokie, IL, USA); IRM (Dentsply Caulk, Milford, DE, USA); Sealer 26 (Dentsply, Petrópolis, RJ, Brazil); and amalgam (Dispersalloy, Dentsply, Petrópolis, RJ, Brazil). All materials were prepared following the manufacturers´ recommendations, with the exception of Portland cement which was prepared in a manner similarly to MTA, and Sealer 26 which was prepared with a thicker consistency than recommended by the manufacturer for root canal filling (4:1, v:v, powder:resin ratio).

Bacterial strains used were as follows: *Porphyromonas gingivalis* (ATCC 33277); *Parvimonas micra* (ATCC 33270); *Fusobacterium nucleatum* subsp. *polymorphum* (ATCC 10953); *Actinomyces viscosus* (own isolate); *Propionibacterium acnes* (own isolate); *Eubacterium saburreum* (ATCC 33271); and *Enterococcus faecalis* (ATCC 29212).

After activation from stock cultures, lyophilized or frozen in skim milk, the bacterial strains were maintained in appropriated culture media until used.

Overnight cultures of the microorganisms were used for inoculum. Facultative bacteria were grown in trypticase-soy broth (TSB) (Difco, Detroit, MI, USA), and anaerobes were grown in pre-reduced anaerobically sterilized brain heart infusion broth (BHI-PRAS) (Difco) supplemented with hemin (5 µg/ml) and vitamin K1 (1 µg/ml). The turbidity of the inoculum, prepared in BHI-PRAS for anaerobes or in TSB for other microorganisms, was adjusted to the turbidity of a 0.5 McFarland BaSO$_4$ standard (approximately 1.5 x 10^8 colony forming units/ml).

Petri dishes containing Brucella agar (Difco) enriched with 5% defibrinated sheep blood and supplemented with hemin and vitamin K1 were seeded with the anaerobic bacteria. Plates containing trypticase soy agar were seeded with the facultative bacteria. Seeding was performed using sterile cotton-tipped applicators which were brushed across the agar surfaces. Six wells of 5 mm depth and 6 mm diameter were punched in each agar plate and filled with freshly prepared sealers. Plates were then left at room temperature for approximately 10 minutes in order to allow for the absorption of the inoculum. All procedures were done in triplicate.

Agar plates inoculated with anaerobic bacteria were incubated in anaerobic jars at 37ºC for 5 days. Anaerobic conditions were obtained by using the GasPak Plus generators (BBL, Becton-Dickinson Microbiology Systems, Cockeysville, MD, USA). Agar plates inoculated with facultative bacteria were incubated aerobically at 37ºC for 24 to 48 hours. The antimicrobial effects of each material were determined by measuring the diameters of the zones of inhibition in millimeters. A diameter of 6 mm served as the cut-off value.

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[*] Jedinice sposobne stvoriti kolonije

Rezultati

Srednje vrijednosti promjera zona bakterijske inhibicije svakog punila za svaku bakterijsku vrstu prikazane su u Tablici 1. Sealer 26 imao je najveće vrijednosti za sve vrste testiranih bakterija (srednja vrijednost 8,1 mm). IRM i Super-EBA spriječili su rast samo *P. gingivalis*, ali s manjim inhibicijskim zonama nego Sealer 26. MTA, Portland cement i amalgam pokazali su nedjelotvorna inhibitorna svojstva kod svih testiranih vrsta bakterija.

Results

The mean diameters of the zones of bacterial inhibition for each sealer against each bacterial species are given in Table 1. Sealer 26 showed the largest inhibition zones of all the species tested (mean 8.1 mm). IRM and Super-EBA were inhibitory exclusively to *P. gingivalis* even with inhibition zones smaller than those from Sealer 26. MTA, Portland cement, and amalgam proved to be ineffective against all the species tested.

Tablica 1 Srednje vrijednosti promjera zone bakterijske inhibicije uzrokovane materijalima za retrogradno punjenje (u milimetrima)
Table 1 Means of the diameters of the zones of bacterial inhibition promoted by materials used for root-end fillings (in mm)

Materials	*P. gingivalis*	*P. micra*	*F. nucleatum*	*A. viscosus*	*P. acnes*	*E. saburreum*	*E. faecalis*	**Mean**
MTA	0	0	0	0	0	0	0	**0**
Portland	0	0	0	0	0	0	0	**0**
Super-EBA	5	0	0	0	0	0	0	**0.7**
IRM	6	0	0	0	0	0	0	**0.9**
Sealer 26	10	6	12	5	5	9	10	**8.1**
Amalgam	0	0	0	0	0	0	0	**0**

Rasprava

Test difuzije u agru bila je metoda izbora u ovom istraživanju kako bi se istražio antibakterijski učinak materijala za retrogradno punjenje. Taj način uobičajen je kod istraživanja antibakterijske učinkovitosti stomatoloških materijala (6-11). Test difuzije u agru ima nekoliko ograničenja. Jedno od njih je da ne pravi razliku između bakteriostatskog i baktericidnog učinka stomatološkog materijala (12). Osim toga, zone inhibicije u agru ne ovise samo o inhibitornom učinku stomatološkog materijala nego test ovisi i o mogućnosti stomatološkog materijala da difundira, širi se kroz agar (8). Osim toga čimbenici kao što su veličina inokuluma, vrijeme inkubacije te dobar kontakt između materijala i agra, također utječu na rezultate (13). Ako pretpostavimo da se većina spomenutih varijabli može uspješno kontrolirati, kod istraživanja u sličnim uvjetima mogu se dobiti konzistentni i mogući rezultati te usporediti antibakterijska svojstva materijala (12, 13). Ipak, kao i kod svakog drugog istraživanja in vitro, rezultate treba vrlo kritički i jako oprezno primjenjivati u kliničkoj praksi.

Materijali za retrogradno punjenje moraju dobro zabrtviti korijenski kanal i tako spriječiti prodor tekućine i bakterija. Osim tih svojstava, moraju imati i antibakterijska kako bi se spriječio daljnji rast bakterija te trebaju baktericidno djelovati na preostale bakterije u kanalu (11). Materijali za retrogradno punjenje trebali bi, osim antibakterijskih svojstava, imati i napredna svojstva mikro-pečaćenja kako bi eliminirali potencijalne bakterije u mikro pukotinama. Od svih materijala testiranih u ovom istraživanju, Sealer 26 pokazao je najbolja antibakterijska svojstva, kad je riječ o svim bakterijskim sojevima. Rezultati istraživanja potvrđuju širok spektar antibakterijske aktivnosti Sealera 26, materijala dokazanog i u drugim studijama (13, 14). Unatoč kalcijevu hidroksidu, antibakterijska svojstva Sealera 26 temelje se na djelovanju hexamethlylene tetramina koji se u vodenastoj otopini raščlanjuje na formaldehid i amonijak, od kojih formaldehid ima izvrsna antimikrobna svojstva.

Discussion

The agar diffusion test was the method of choice in this study to investigate the antibacterial activity of materials used in retro-fillings. This method has been widely used to evaluate the antibacterial activity of dental materials (6-11). The agar diffusion test has some limitations since it cannot distinguish between bacteriostatic and bactericidal effects (12), and the zones of inhibition are not only related to the inhibitory effects of the material, but also depend on the diffusibility of the material across the medium (8). Moreover, factors such as inoculum size, incubation time, and good material/agar contact may also interfere with the results (13). However, if most of these variables are properly controlled, consistent and reproducible results may be obtained and, subsequently, materials can be compared for antibacterial effects under similar test conditions (12,13). Nevertheless, as with any other in vitro test, the results of this method can only be directly extrapolated to the clinical setting with caution.

In addition to having the capacity to provide a fluid- and bacteria-tight seal of the root canal, the retro-filling material should have antibacterial effects in order to kill residual bacteria in the retrograde cavity and inhibit further bacterial growth (11). A retro-filling material with antibacterial activity may also possess an enhanced bacteria-tight sealing ability because bacteria along occasional microleakage channels can be eliminated by the material.

Out of the materials examined in this study, Sealer 26 demonstrated antibacterial activity against all species tested and for each, its performance surpassed that of the other test materials. This confirms Sealer 26´s broad antibacterial activity reported by other studies (13,14). Despite the presence of calcium hydroxide in its formulation, the antibacterial effectiveness of this cement is attributed primarily to the presence of hexamethylene tetramine, which in aqueous solution decomposes into formaldehyde and ammonia, the former being an excellent antimicrobial agent.

IRM i Super-EBA antibakterijski su djelovali samo na *Porphyromonas gingivalis* koji se može naći u ponekim slučajevima perzistentnih ili sekundarnih infekcija kod endodontski liječenih zuba. Iako su materijali za punjenje na bazi cinkova oksida/eugenola snažno antibakterijski aktivni (9, 13, 16), cementi Super EBA i IRM gušće se pripremaju negoli cementi od cinkova oksida/eugenola te je tako smanjena mogućnost otpuštanja eugenola i posljedično antibakterijsko svojstvo punila.

Kod MTA, cementa Portland i amalgama nisu potvrđena antibakterijska svojstva na testiranim sojevima bakterija. MTA i cement Portland sličnog su sastava (17), što objašnjava i slične rezultate. Za razliku od rezultata u ovom istraživanju, u ostalim istraživanjima istaknuto je da MTA antimikrobno djeluje na gljivicu *Candida albicans* (18-20) i određene fakultativno anaerobne bakterije (21). Ta razlika u rezultatima može se pripisati razlikama u postupcima istraživanja. No, rezultati naših istraživanja podudaraju se s rezultatima u nekim ranijim istovrsnim istraživanjima. Torabinejad i suradnici (11) dokazali su da MTA ne pokazuje antimikrobna svojstva kad je riječ o anaerobnim bakterijama i *E. faecalis*. Estrela i njegovi kolege (22) pokazali su da MTA i cement Portland nemaju antimikrobna svojstva u slučaju fakultativnih anaeroba i anaeroba te *C. albicans*. Miyagak i suradnici (23) istaknuli su da MTA i cement Portland nemaju antimikrobni učinak na *C. albicans*, *Staphylococcus aureus* i *Escherichia coli*. U jednoj drugoj studiji *in vitro* (24) kaže se da su MTA i cement Portland pokazali inhibitorna svojstva na *P. aeruginosa*, ali to sprječavanje nije uočeno kada su u pitanju bili *E. faecalis* i *E. coli*. Važno je istaknuti kako je moguće da su svojstva MTA i cementa Portland promijenjena zbog utjecaja puferskog sustava bakterijskog medija, ali to se treba istražiti. Neki autori kod pripreme MTA sugeriraju uporabu 2-postotnog klorheksidina umjesto destilirane vode, kako si se poboljšala antimikrobna svojstva (25, 26). Iako su mu antibakterijska svojstva upitna, MTA se vrlo često i uspješno rabi kao materijal za retrogradno punjenje (27).

Kad je riječ o amalgamu, u dosadašnjim istraživanjima (7-11) nije dokazana antimikrobna aktivnost te su u skladu s trenutačnim rezultatima. Ovi rezultati nisu u skladu s istraživanjem Eldeniza i njegovih kolega (21) u kojemu je dokazano antimikrobno svojstvo amalgama kada je u izravnom doticaju s fakultativnim anaerobnim bakterijama. Treba isto tako istaknuti da razlike u metodama i bakterijskim uzorcima mogu dati različite rezultate. Pozitivni učinci korištenja amalgama za retrogradno punjenje upitni su zbog loših antimikrobnih svojstava, slabog brtvljenja i korozije te neprihvatljivih fizičko-kemijskih svojstava (28, 29).

Zaključak

Sudeći prema rezultatima istraživanja i uzimajući u obzir ograničenja u metodama istraživanja, možemo zaključiti da je Sealer 26 pokazao izvrstan antibakterijski učinak u usporedbi s ostalim često korištenim materijalima za retrogradno

Both IRM and Super-EBA exhibited antibacterial activity only against *Porphyromonas gingivalis*, which can be found in certain cases of persistent/secondary infection associated with root canal-treated teeth (15). Although zinc oxide/eugenol sealers have been demonstrated to exhibit pronounced antibacterial activities (9,13,16), Super EBA and IRM cements are prepared in thicker consistencies than zinc oxide/eugenol sealers used for root canal filling, thereby reducing the amount of eugenol available to exert antibacterial activity. Another reason for differences may be attributed to the experimental method and the bacterial species used.

MTA, Portland cement, and amalgam showed no antibacterial activity against the species tested. Both MTA and Portland cement have a similar composition (17), which explains the similar results obtained for these materials. In contrast to our findings, other studies have found MTA to demonstrate effective antimicrobial action against the yeast *Candida albicans* (18-20) and certain facultative bacteria (21). This divergence may be attributed to methodological differences. However, our findings are in agreement with several other studies on the subject. As for MTA, Torabinejad *et al.* (11) found no antimicrobial activity for this material against anaerobic bacteria and *E. faecalis*. Estrela *et al.* (22) also reported no antimicrobial activity for MTA and Portland cement against facultative and aerobic bacteria, as well as *C. albicans*. Miyagak et al. (23) observed no antimicrobial effects for both MTA and Portland cement on *C. albicans*, *Staphylococcus aureus*, and *Escherichia coli*. In another *in vitro* study (24), although both materials were inhibitory to *P. aeruginosa*, no effects were reported against *E. faecalis* and *E. coli*. It is relevant to point out that the antibacterial effects of both MTA and Portland cement may have been influenced by the buffer ability of the culture medium, but this point remains to be clarified. Some authors have suggested the addition of 2% chlorhexidine rather than distilled water to MTA in an attempt to improve its antimicrobial activity (25,26). Albeit its antimicrobial action is somewhat controversial, MTA has been widely used as root-end filling material with a high success rate (27).

With regards to amalgam, the present findings are in line with other previous studies (7-11), which also showed no significant antimicrobial effects for this material. This finding disagrees with Eldeniz *et al.* (21), who observed antimicrobial activity for amalgam through the direct contact test against some facultative bacteria. It should be emphasized that the dissimilar results may be explained by the differences in methodology and bacterial species/strains used. The utilization of amalgam as root-end filling material has been questioned not only because of the lack of significant antimicrobial activity but also because its physicochemical properties, such as poor sealing ability and corrosion, are inadequate for retro-fillings (28, 29).

Conclusion

According to the present findings and considering methodological limitations, it can be concluded that, amongst the materials tested, Sealer 26 exhibited more pronounced antibacterial effects when compared to other commonly used

punjenje. Buduća istraživanja potrebna su kako bi se detaljnije analizirali učinci Sealera 26 u kliničkim uvjetima.

root-end filling materials. Further studies using other methods and clinical trials are required to thoroughly analyze the performance of this material in root-end fillings.

Zahvale

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Abstract
Purpose: The present study was undertaken to evaluate and compare the antibacterial effectiveness of five root-end filling materials. **Material and methods:** The agar diffusion method was employed to evaluate the inhibition zone provided by the following root-end filling materials: Pro Root MTA, Portland cement, Super-EBA, IRM, Sealer 26, and silver amalgam. The target microorganisms included five anaerobic bacterial species (*Porphyromonas gingivalis*, *Parvimonas micra*, *Fusobacterium nucleatum*, *Propionibacterium acnes* and *Eubacterium saburreum*) and two facultative bacterial species (*Actinomyces viscosus* and *Enterococcus faecalis*). **Results:** Sealer 26 demonstrated the largest inhibitory effects on all species tested. IRM and Super-EBA were inhibitory only to *P. gingivalis*, but this effect was far less pronounced as compared to Sealer 26. MTA, Portland cement, and amalgam proved ineffective against all species tested. **Conclusion:** These findings revealed that Sealer 26 has more pronounced antibacterial effects when compared to other commonly used root-end filling materials.

References

1. Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surg Oral Med Oral Pathol. 1965 Sep;20:340-9.
2. Siqueira JF Jr, Rôças IN. Clinical implications and microbiology of bacterial persistence after treatment procedures. J Endod. 2008 Nov;34(11):1291-1301.
3. Tronstad L, Sunde PT. The evolving new understanding of endodontic infections. Endod Topics. 2003; 6(1):57-77.
4. Gartner AH, Dorn SO. Advances in endodontic surgery. Dent Clin North Am. 1992 Apr;36(2):357-78.
5. Torabinejad M, Pitt Ford TR. Root end filling materials: a review. Endod Dent Traumatol. 1996 Aug;12(4):161-78.
6. Orstavik D. Antibacterial properties of root canal sealers, cements and pastes. Int Endod J. 1981 May;14(2):125-33.
7. Chong BS, Owadally ID, Pitt Ford TR, Wilson RF. Antibacterial activity of potential retrograde root filling materials. Endod Dent Traumatol. 1994 Apr;10(2):66-70.
8. Fraga RC, Siqueira JF Jr, de Uzeda M. In vitro evaluation of antibacterial effects of photo-cured glass ionomer liners and dentin bonding agents during setting. J Prosthet Dent. 1996 Nov;76(5):483-6.
9. Siqueira Jr JF, Gonçalves RB. Antibacterial activities of root canal sealers against selected anaerobic bacteria. J Endod 1996; 22(2):79-80.
10. Shalhav M, Fuss Z, Weiss EI. In vitro antibacterial activity of a glass ionomer endodontic sealer. J Endod. 1997 Oct;23(10):616-9.
11. Torabinejad M, Hong CU, Pitt Ford TR, Kettering JD. Antibacterial effects of some root end filling materials. J Endod. 1995 Aug;21(8):403-6.
12. Tobias RS. Antibacterial properties of dental restorative materials: a review. Int Endod J. 1988 Mar;21(2):155-60.
13. Siqueira JF Jr, Favieri A, Gahyva SM, Moraes SR, Lima KC, Lopes HP. Antimicrobial activity and flow rate of newer and established root canal sealers. J Endod. 2000 May;26(5):274-7.
14. Tanomaru-Filho M, Tanomaru JM, Barros DB, Watanabe E, Ito IY. In vitro antimicrobial activity of endodontic sealers, MTA-based cements and Portland cement. J Oral Sci. 2007 Mar;49(1):41-5.
15. Siqueira JF Jr, Rôças IN. Polymerase chain reaction-based analysis of microorganisms associated with failed endodontic treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2004 Jan;97(1):85-94.
16. al-Khatib ZZ, Baum RH, Morse DR, Yesilsoy C, Bhambhani S, Furst ML. The antimicrobial effect of various endodontic sealers. Oral Surg Oral Med Oral Pathol. 1990 Dec;70(6):784-90.
17. Sipert CR, Hussne RP, Nishiyama CK, Torres SA. In vitro antimicrobial activity of Fill Canal, Sealapex, Mineral Trioxide Aggregate, Portland cement and EndoRez. Int Endod J. 2005 Aug;38(8):539-43.
18. Al-Nazhan S, Al-Judai A. Evaluation of antifungal activity of mineral trioxide aggregate. J Endod 2003; 29:826-827.
19. Al-Hezaimi K, Al-Hamdan K, Naghshbandi J, Oglesby S, Simon JH, Rotstein I. Effect of white-colored mineral trioxide aggregate in different concentrations on Candida albicans in vitro. J Endod. 2005 Sep;31(9):684-6.
20. Al-Hezaimi K, Naghshbandi J, Oglesby S, Simon JH, Rotstein I. Comparison of antifungal activity of white-colored and gray-colored mineral trioxide aggregate (MTA) at similar concentrations against Candida albicans. J Endod. 2006 Apr;32(4):365-7.
21. Eldeniz AU, Hadimli HH, Ataoglu H, Orstavik D. Antibacterial effect of selected root-end filling materials. J Endod. 2006 Apr;32(4):345-9.
22. Estrela C, Bammann LL, Estrela CR, Silva RS, Pécora JD. Antimicrobial and chemical study of MTA, Portland cement, calcium hydroxide paste, Sealapex and Dycal. Braz Dent J. 2000;11(1):3-9.
23. Miyagak DC, de Carvalho EM, Robazza CR, Chavasco JK, Levorato GL. In vitro evaluation of the antimicrobial activity of endodontic sealers. Braz Oral Res. 2006 Oct-Dec;20(4):303-6.
24. Ribeiro CS, Kuteken FA, Hirata Júnior R, Scelza MF. Comparative evaluation of antimicrobial action of MTA, calcium hydroxide and Portland cement. J Appl Oral Sci. 2006 Oct;14(5):330-3.
25. Stowe TJ, Sedgley CM, Stowe B, Fenno JC. The effects of chlorhexidine gluconate (0.12%) on the antimicrobial properties of tooth-colored ProRoot mineral trioxide aggregate. J Endod. 2004 Jun;30(6):429-31.
26. Holt DM, Watts JD, Beeson TJ, Kirkpatrick TC, Rutledge RE. The anti-microbial effect against enterococcus faecalis and the compressive strength of two types of mineral trioxide aggregate mixed with sterile water or 2% chlorhexidine liquid. J Endod. 2007 Jul;33(7):844-7.
27. Christiansen R, Kirkevang LL, Hørsted-Bindslev P, Wenzel A. Randomized clinical trial of root-end resection followed by root-end filling with mineral trioxide aggregate or smoothing of the orthograde gutta-percha root filling--1-year follow-up. Int Endod J. 2009 Feb;42(2):105-14.
28. Dorn SO, Gartner AH. Retrograde filling materials: a retrospective success-failure study of amalgam, EBA, and IRM. J Endod. 1990 Aug;16(8):391-3.
29. Chong BS, Pitt Ford TR, Watson TF, Wilson RF. Sealing ability of potential retrograde root filling materials. Endod Dent Traumatol. 1995 Dec;11(6):264-9.