J Stoma 2016; 69, 5: 521-530
DOI: 10.5604/00114553.1230106

ORIGINAL ARTICLE

In vitro evaluation of the antibacterial effect of various root canal sealers on selected anaerobic bacteria*

Ocena przeciwbakteryjnego działania różnych uszczelniaczy kanałowych na wybrane szczepy bakterii beztlenowych – badania w warunkach *in vitro**

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Abstract

Aim of the study. *To determine and compare the antibacterial activity of various root canal sealers against selected strains of anaerobic bacteria: Fusobacterium nucleatum ATCC 25586, Porphyromonas gingivalis ATCC 33277, Peptostreptococcus anaerobius ATCC 27337.* ***Material and Methods****. The materials tested in this study included AH Plus Jet (AH), Apexit Plus (AP), Endomethasone N (EN), Epiphany (EP), GuttaFlow (GF), Roeko Seal Automix (RSA), Sealapex (SP) and Tubliseal (TS). The antibacterial effect of the freshly mixed sealers on the above mentioned strains of bacteria was evaluated with the use of the agar diffusion test. After inoculation with the bacteria and applying the materials, the agar plates were incubated at 37°C for one week in an atmosphere of 5-10% CO_2. The results were obtained*

Streszczenie

Cel pracy*. Ocena i porównanie antybakteryjnej aktywności różnych uszczelniaczy kanałowych względem wybranych szczepów bakterii beztlenowych: Fusobacterium nucleatum ATCC 25586, Porphyromonas gingivalis ATCC 33277, Peptostreptococcus anaerobius ATCC 27337.* ***Materiał i metody.*** *Badania przeprowadzono z użyciem następujących materiałów: AH Plus Jet (AH), Apexit Plus (AP), Endomethasone N (EN), Epiphany (EP), GuttaFlow (GF), Roeko Seal Automix (RSA), Sealapex (SP) i Tubliseal (TS). Działanie antybakteryjne świeżo zarobionych uszczelniaczy na wyżej wymienione szczepy bakterii badano z zastosowaniem metody dyfuzji agarowej. Po rozprowadzeniu zawiesiny bakteryjnej i aplikacji materiałów, płytki inkubowano w temperaturze 37°C przez 1 tydzień w atmosferze 5-10%*

KEYWORDS:
root canal sealers, antibacterial activity, agar diffusion test

HASŁA INDEKSOWE:
uszczelniacze kanałowe, aktywność przeciwbakteryjna, test dyfuzji agarowej

* The work was supported by the Medical University of Bialystok grant No: 123-09832L.
* Praca zrealizowana w ramach badawczego projektu statutowego UMB nr: 123-09832.

by measuring the diameter of the growth inhibition zone after 48 h, 72 h, one week (F. nucleatum) or 72 h, 120 h and one week (P. gingivalis, P. anaerobius), and analyzed statistically using one-way analysis of variance (ANOVA) followed by post-hoc Tukey's test. ***Results.*** *AH, EN, EP, SP and TS significantly inhibited all the bacterial strains over the experimental period. AP showed a slight suppressive effect on P. gingivalis only. No antimicrobial activity was detected for GF and RSA.* ***Conclusions****. The antimicrobial effect of root canal sealers varied considerably depending on the type of material and the tested bacterial species. Most of the sealers demonstrated antibacterial action on the investigated bacteria.*

CO_2. Wyniki odczytywano mierząc strefy zahamowania wzrostu bakteryjnego po 48 h, 72 h, 1 tygodniu (F. nucleatum) lub 72 h, 120 h i 1 tygodniu (P. gingivalis, P. anaerobius), a następnie analizowano statystycznie z zastosowaniem jednoczynnikowej analizy wariancji ANOVA i testu post-hoc Tukey'a. ***Wyniki.*** *AH, EN, EP, SP i TS znacząco hamowały wszystkie badane szczepy przez cały okres obserwacji. AP wykazał nieznaczne działanie hamujące tylko na P. gingivalis. Brak antybakteryjnej aktywności stwierdzono w przypadku RSA i GF.* ***Wnioski.*** *Przeciwdrobnoustrojowe działanie uszczelniaczy różniło się znacznie w zależności od rodzaju materiału i badanego szczepu bakteryjnego. Większość uszczelniaczy kanałowych wykazywała przeciwbakteryjną aktywność wobec badanych szczepów.*

Introduction

Microorganisms invading and colonizing the root canal system are the major etiologic agents of periradicular disease.[1] Endodontic milieu with the necrotic pulp and low oxygen tension mainly supports the development of anaerobic microflora. Particular bacterial strains do not exist in the root canal as free-floating bacteria (planktonic microorganisms), but grow in the extracellular polysaccharide-protein matrix forming biofilms – well-organized, co-operating adherent microbial communities.[2]

Gram-negative anaerobic rods, such as *Fusobacterium nucleatum, Porphyromonas gingivalis,* and Gram-positive cocci as *Peptostreptococcus species*, are frequently isolated from the root canals of untreated teeth as well as filled roots with periapical lesions.[3-7]

Fusobacterium nucleatum is one of the most important anaerobic Gram-negative bacterium that can be closely associated with endodontic flare-ups. It is well known that lipopolysaccharide (LPS) contained in its cell wall is an endotoxin involved in the initiation of periapical inflammation and alveolar bone destruction.[5,7] *Yamasaki* et al.[8] demonstrated that *F. nucleatum* had a cytotoxic potential in periapical fibroblast cultures, and inhibited their growth and proliferation. In addition, this bacterium mediates significant aggregation of immune cells, which is directly correlated with induction of cell death.[9]

Moreover, *F. nucleatum* is regarded as a bridging organism in root canal biofilm due to some protein adhesins contained in the cell membranes, "bridging" together species which otherwise would not interact.[10] It has been shown that this bacterium communicates with other microorganisms in the biofilm, e.g. *Porphyromonas gingivalis, Peptostreptococcus micros*, using a bacterial cell-to-cell communication mechanism (quorum sensing) for controlling cellular functions. It is known to be involved in the regulation of several microbial properties including virulence and resistance to anti-microbial agents, and therefore may increase the pathogenic potential of bacteria.[5,8]

Porphyromonas gingivalis is characterized by significant virulence due to a large number of putative virulence determinants such as lipopolysaccharide, fimbriae, capsule, lipoproteins and others.[3] They can provoke inflammatory reactions, initiate tissue destruction and even perturb host defense mechanisms. Moreover, *P. gingivalis* is capable of resisting neutrophil phagocytosis by degradation of enzymes released from host defense cells, and also some immunoglobulins and complement factors.[3] *P. gingivalis* is also able to initiate degradation of serum proteins and thus enable the growth of *Fusobacterium* and *Peptostreptococcus*, which cannot hydrolyze intact proteins despite producing peptidases.[11] It would seem, therefore, that

Table 1. Compositions of materials tested for antibacterial activity

Name	Source	Active ingredients
AH Plus Jet™ (AH)	Dentsply DeTrey GmbH, Konstanz, Germany	Bisphenol-A epoxy resin, Bisphenol-F epoxy resin, Calcium tungstate, Zirconium oxide, silica, iron oxide pigments, dibenzyldiamine, aminoadamantane, tricyclodecane-diamine, silicone oil
Apexit® Plus (AP)	Ivoclar Vivadent AG, Schaan, Lichtenstein	Calcium salts (hydroxide, oxide, phosphate), hydrogenised colophony, disalicylate, bismuth salts (oxide, carbonate), highly dispersed silicon dioxide, alkyl ester of phosphoric acid
Endomethasone N (EN)	Septodont, Cedex, France	Zinc oxide, hydrocortisone acetate, thymol iodide, barium sulfate, magnesium stearate
Eugenol	Chema – Elektromet, Rzeszów, Poland	eugenol
Epiphany (EP)	Pentron® Clinical Technologies, LLC Wallingford CT, USA	Organic part: BisGMA, ethoxylated BisGma, UDMA, hydrophilic difunctional methacrylates Inorganic part: calcium hydroxide, barium sulphate, barium glass, bismuth oxychloride, silica
Gutta-Flow® (GF)	Coltene/Whaledent GmbH+Co. KG, Langenau, Germany	Gutta-percha powder, polidimethylosiloxane, silicone oil, platin catalyst, zirconium dioxide, nano-silver, coloring
Roeko Seal Automix (RSA)	Coltene/Whaledent GmbH+Co. KG, Langenau, Germany	Polydimethylsiloxane, silicone oil, paraffin-base oil, platinum catalyst, zirconium dioxide
Sealapex (SP)	Kerr Italia S.p.A., Salerno, Italy	Calcium oxide, bismuth trioxide, zinc oxide, sub-micron silica, 2% titanium dioxide, zinc stearate, tricalcium phosphate, blend
Tubli-Seal (TS)	Kerr Italia S.p.A., Salerno, Italy	Zinc oxide, barium sulfate, oleo resin, oils/modifiers, thymol iodide, eugenol

coexistence and synergistic associations with *P. gingivalis, F. nucleatum,* and *Peptostreptococcus* in root canal biofilm may constitute contributing agents in apical periodontitis.[5,12]

The ultimate goal of endodontic treatment, consisting of chemomechanical preparation and three-dimensional obturation of the root canal space, is the eradication of bacteria from the root canal system and prevention of subsequent reinfection. This enables achieving optimal conditions for healing of inflamed periradicular tissues after root canal treatment.[1]

It is well recognized that the huge majority of bacteria are sensitive to standard treatment procedures. Nevertheless, certain Gram-negative anaerobic species such as *F. nucleatum, Prevotella* and especially Gram-positive anaerobic cocci can be detected in post-instrumentation samples.[1]

Consequently, the use of root canal sealer with antibacterial activity may be advantageous in an effort to further eliminate residual microorganisms that have survived chemomechanical instrumentation, as well as to prevent recurrent reinfections.[13-15]

Thus, the aim of this *in vitro* study was to evaluate and compare the antimicrobial activity of currently used root canal sealers on three common endodontic pathogens: *Fusobacterium nucleatum, Porphyromonas gingivalis,* and *Peptostreptococcus anaerobius.*

Material and methods

Table 1 presents the materials used for the experiment and their composition. The standardized *Peptostreptococcus anaerobius ATCC 27337, Fusobacterium nucleatum ATCC*

25586, Porphyromonas gingivalis ATCC 33277 were obtained from Microbiologics, Inc., St. Cloud, Minnesota, USA. The strains were cultured on Schaedler agar (Emapol sp. z o.o., Gdańsk, Poland) supplemented with 5% of sheep blood and incubated for 24-72 hours at 37^0C under anaerobic conditions. The antibacterial activity of root canal sealers against the standardized strains of anaerobic bacteria was determined using the agar diffusion method on Brucella Blood Agar (Oxoid Limited, Hampshire, United Kingdom) enriched with 5% of sheep blood, vitamin K_1 and 1% of hemin. After 24-72 h, suspensions of bacterial strains in the Brucella broth of 1.0 of turbidity on the McFarland scale were prepared.

The bacterial suspension was distributed with a sterile cotton swab on the surface of Brucella Blood Agar with wells of 7 mm in diameter and 5 mm in depth. In order to seal up the wells, the bottom was covered with 10 µl of liquid Triptic Soya Agar (TSA) (Oxoid Limited, Hampshire, United Kingdom), and the wells were filled with freshly mixed sealers prepared in aseptic conditions according to the manufacturer's instructions. The plates were left in room temperature for 30 min. and then incubated for 48-72 hours in anaerobic conditions at 37^0C. To control the growth of the standardized strain on the agar used for the experiment, positive control plates were streaked with bacteria, but no root canal sealer was used. Six samples were prepared for each material.

The plates were examined and evaluated for growth inhibition zones at 48 hours, 72 hours, and 7 days *(F.nucleatum*) or at 72 hours, 120 hours, 7 days *(P. gingivalis, P. anaerobius)*. The most uniform segment at the largest point of the growth inhibition zone was measured with a ruler and the results were given in millimetres. The 7 mm (diameter of the well) was extracted from the measurement as the cut-off value.[15,16] Wider zones of inhibition were interpreted to indicate greater antimicrobial activity of the involved materials.

Statistical analysis was performed using the Statistica 8.0 (StatSoft) software package. The collected data were statistically analyzed by comparing mean inhibition zone for each sealer. The obtained results were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's test and non-parametric Spearman's correlation coefficient. The level of significance was set at $p< 0.05$.

Results

The growth inhibition zones resulting from the application of different root canal sealers on the tested bacteria are shown in Figures 1-3. Most of the sealers (EN, SP, TS AH, and EP) showed antimicrobial activity on all tested bacterial strains. AP demonstrated antibacterial action only against *P. gingivalis*. GF and RSA were ineffective against all bacteria. Positive control plates showed bacterial growth in all cases. Analysis of variance revealed that the mean bacterial growth inhibition zone was significantly influenced by the tested material type ($p< 0.001$).

EN, followed by SP, TS, AH, AP and EP, exhibited the highest antibacterial effect against *P. gingivalis*. In relation to *F. nucleatum*, the average dimension of growth inhibition zones in descending order were detected with SP, TS, EN, AH, and EP. The largest growth inhibition zones for *P. anaerobius* were observed with EN and EP followed by TS, SP, and AH.

Statistical analysis of the results revealed significant differences between all the materials in the varying sizes of growth inhibition zones within particular strains. Details of the statistical analysis results are presented in Figures 1-3.

In order to compare the susceptibility of particular strains to each sealer, the mean growth inhibition zones obtained with all the measurements for each material (excluding materials with a zone of growth inhibition 0) were calculated and analyzed. Statistical analysis revealed that *P. gingivalis* was significantly more susceptible than *F. nucleatum* and *P. anaerobius* to AH, AP, and EN ($p<0.001$) and more susceptible than *P. anaerobius* to almost all tested sealers (except for EP). On the other hand, *P. anaerobius* was significantly more susceptible than *F. nucleatum* and *P. gingivalis* to EP ($p<0.001$). *F. nucleatum* was significantly more susceptible than *P. anaerobius* to AH, SP, and TS ($p<0.001$) (Table 2).

The antibacterial effect of all the tested root

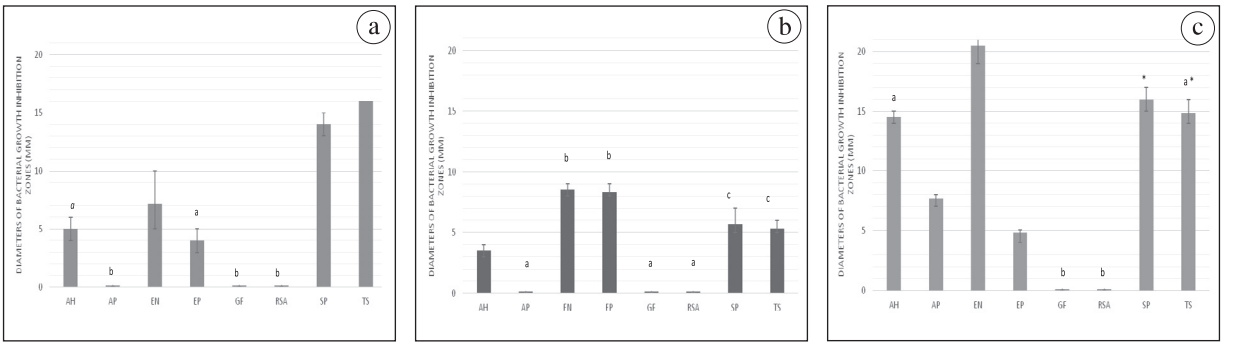

Fig. 1. *Zones of bacterial growth inhibition in millimeters provided by root canal sealers; a – F. nucleatum at 48 hours, b – P. gingivalis at 72 hours, c – P. anaerobius at 72 hours.*
All the values which have not been tagged with identical lowercase and symbols show statistically significant differences at a level of p <0.001;
*The values which have been tagged with * are statistically significant at a level of p<0.01;*
The values which have been tagged with identical lowercase [a,b,c] are not statistically significant (p>0.05).

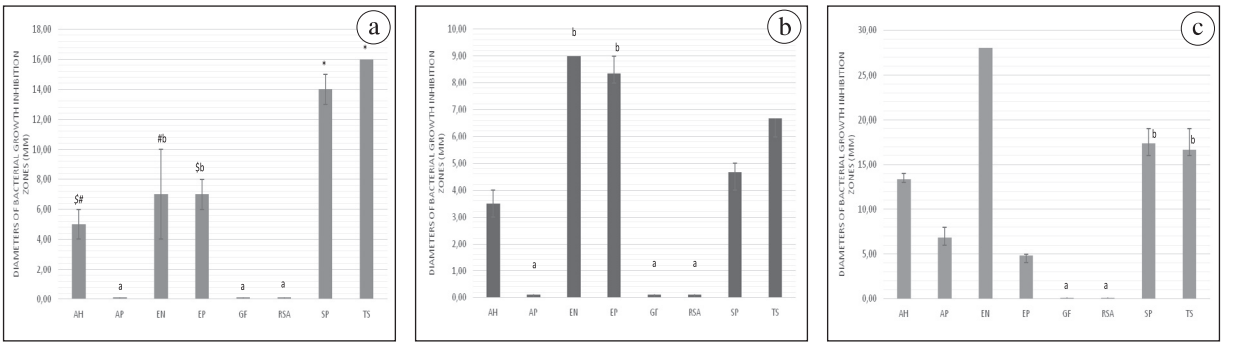

Fig. 2. *Zones of bacterial growth inhibition in millimeters provided by root canal sealers; a – F. nucleatum at 72 hours, b – P. gingivalis at 120 hours, c – P. anaerobius at 120 hours.*
All the values which have not been tagged with identical lowercase and symbols show statistically significant differences at a level of p <0.001;
*The values which have been tagged with *$# are statistically significant at a level of p<0.01;*
The values which have been tagged with identical lowercase [a,b] are not statistically significant (p>0.05).

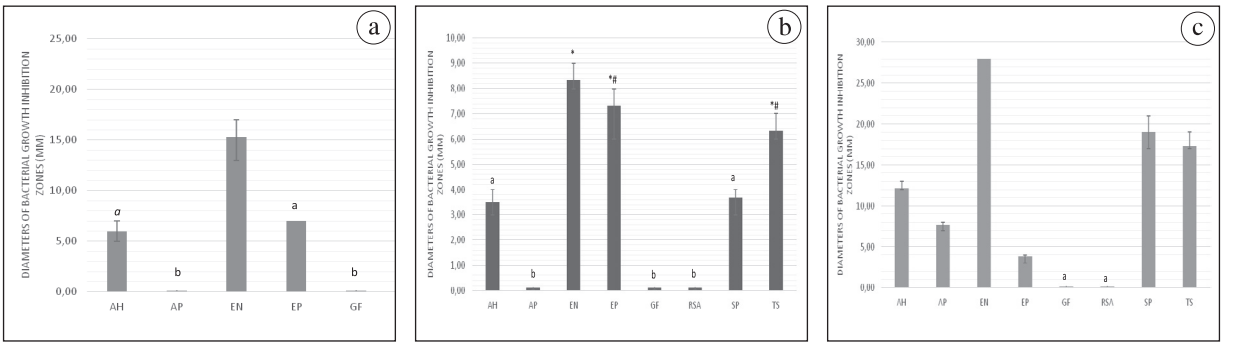

Fig. 3. *Zones of bacterial growth inhibition in millimeters provided by root canal sealers at 7 days; a – F. nucleatum, b – P. gingivalis, c – P. anaerobius.*
All the values which have not been tagged with identical lowercase and symbols show statistically significant differences at a level of p <0.001;
*The values which have been tagged with *# are statistically significant at a level of p<0.01;*
The values which have been tagged with identical lowercase [a,b] are not statistically significant (p>0.05).

Table 2. Mean bacterial growth inhibition zones from all periods of observations for each material (excluding materials showing no bacterial growth inhibition zone)

Material	Bacteria strain	Mean	SD	p	
AH	*F. nucleatum*	5.33	0.56	F. n. – P. a	p<0.001
	P. anaerobius	3.50	0.55	F. n. – P. g	
	P. gingivalis	13.33	0.42	P. a. – P. g	
AP	*F. nucleatum*	0	0	F. n. – P. a.	p=1.000
	P. anaerobius	0	0	F. n. – P. g	p<0.001
	P. gingivalis	7.39	0.49	P. a – P. g	p<0.001
EN	*F. nucleatum*	9.83	1.59	F. n. – P. a	p=0.061
	P. anaerobius	8.61	0.25	F. n. – P. g	p<0.001
	P. gingivalis	25.50	0.28	P. a – P. g	p<0.001
EP	*F. nucleatum*	6.00	0.37	F. n. – P. a	p<0.001
	P. anaerobius	8.00	0.56	F. n. – P.g	
	P. gingivalis	4.50	0.41	P. a – P. g	
SP	*F. nucleatum*	17.00	0.42	F. n. – P.a	p<0.001
	P. anaerobius	4.67	0.56	F. n. – P.g	p=0.919
	P. gingivalis	17.44	1.00	P. a – P. g	p<0.001
TS	*F. nucleatum*	17.00	0.21	F. n. – P. a	p<0.001
	P. anaerobius	6.11	0.34	F. n. – P. g	p=0.690
	P. gingivalis	16.28	0.74	P. a – P. g	p<0.001

SD – standard deviation; p – level of significance.

canal sealers against individual strains varied considerably during the entire experiment. For *F. nucleatum*, the antibacterial activity of the materials increased throughout the whole experiment. For *P.gingivalis*, the antibacterial effect of EN, TS, and SP rose, whereas with AH and EP it decreased gradually. The diameter of growth inhibition zones with AP remained stable over the experimental period. For *P. anaerobius*, only TS exhibited an enhancement of antibacterial activity. EN and AH were characterized by a stable increase of the growth inhibition zones, and the antibacterial effect of EP and SP decreased during the experiment.

Table 3 presents the results of correlation between the mean bacterial growth inhibition zones of the materials and the time of the experiment. Statistically significant correlations were found for all microorganisms tested with EP, SP, TS and further for *P. gingivalis* with EN, AH, and also for *F. nucleatum* with EN ($p<0.05$).

Discussion

It has been proven that different bacteria may vary in their sensitivity to the same material.[16] Therefore, it seems important to use more than one species of bacteria to test the antibacterial effect of root canal materials. *Fusobacterium*

Table 3. Spearman's non-parametric correlation between the means of bacterial growth inhibition zones for each material and the time of the experiment (excluding materials showing no bacterial growth inhibition zone)

Microorganism	Material	R	p	Correlation
Fusobacterium nucleatum	AH	0.45	p=0.064	moderate
	EN	0.74	**p=0.001**	**high**
	EP	0.78	**p<0.001**	**high**
	SP	0.76	**p<0.001**	**high**
	TS	0.85	**p<0.001**	**high**
Porphyromonas gingivalis	AH	-0.91	**p<0.001**	**high**
	AP	0.00	1.000	high
	EN	0.85	**p<0.001**	**high**
	EP	-0.69	**p=0.001**	**high**
	SP	0.79	**p=0.001**	**high**
	TS	0.84	**p<0.001**	**high**
Peptostreptococcus anaerobius	AH	0.00	p=1.000	high
	EN	-0.14	p=0.581	high
	EP	-0.54	**p=0.020**	**moderate**
	SP	-0.86	**p<0.001**	**high**
	TS	0.54	**p=0.022**	**moderate**

R – Spearman's rank correlation coefficient; p – level of significance; high correlation r > 0.6; moderate correlation 0.3 < r < 0.6; low correlation r < 0.3.

nucleatum, Porphyromonas gingivalis, and *Peptostreptococcus anaerobius* have been shown to be frequently present in infected root canals and were, therefore, chosen as representatives for our study.[3-7]

It is extremely difficult to compare accurately bacterial inhibition data, even for identical materials, between different authors with the agar diffusion test due to difficulties in controlling the large number of variables. This means that there are no standardized *in vitro* protocols for testing the antimicrobial activity of materials.[14]

The findings of the present study have suggested substantial differences in the antimicrobial effect of root canal sealers. Among the materials tested, Endomethasone N, Tubliseal, AH Plus Jet, Epiphany and Sealapex showed antibacterial activity against all examined strains. These results are in accordance with previous findings evaluating the antibacterial effect of zinc oxide sealers and those based on epoxy resins on anaerobic bacteria.[15-18] The antimicrobial effect of Endomethasone N and Tubliseal is attributed to free eugenol liberated from these sealers even after their setting. Eugenol, a phenolic compound, in relatively high concentrations inhibits the growth, or even induces death of bacterial cells.[19] The adverse effect of AH Plus on bacteria might be related to the content of mutagenic bisphenol diglycidyl ether[19] and epoxy amines.[15] Taken

together, these components made the epoxy resin sealers antimicrobial.

Epiphany, a polymethacrylate resin-based sealer, also inhibited the growth of all the tested bacterial anaerobic strains. The antimicrobial activity of this sealer can be possibly attributed to the toxicity of methacrylates and the release of unreacted and residual monomers.[20,21] On the other hand, several authors have pointed out that the release of free monomers from materials characterized by a high water sorption, and thus high solubility such as Epiphany, might stimulate bacterial growth.[22]

Calcium hydroxide-containing sealers had a varied antibacterial effect. Apexit Plus was effective only on *P. gingivalis,* which is considered one of the most sensitive microorganisms.[6] Other investigators also emphasized the poor antibacterial properties of Apexit Plus.[13,16,17] It can be speculated that the increased pH resulting from the dissociation of calcium hydroxide into calcium and hydroxyl ions could have been responsible for any antimicrobial action of the calcium hydroxide sealers. The hydroxyl ions denature proteins of the cytoplasmatic membrane of bacteria, thus killing the cells.[23] Our previous investigation has shown that Sealapex increased the pH of the environment considerably more than Apexit Plus, and thus potently inhibited the growth of all bacterial strains in the present study.[24] These results are consistent with the findings of other authors.[17,19,25] However, *Ebert* et al.[26] presumed that the pH of calcium hydroxide sealers in culture media may not be high enough to suppress the growth of bacterial strains due to the buffering properties of the agar medium used.

It should be remembered that the antibacterial action of root canal obturative materials might be also modified by dentine.[27] The influence of dentine on the antibacterial activity of certain drugs is associated with a buffering effect of this tissue.[28]

In the present experiment, RSA and GuttaFlow did not show an antibacterial effect against any of the tested microbial strains despite the fact that they contain antimicrobial components such as nano-silver particles (GuttaFlow).[21] *Cobankara* et al.[19] claimed that no antibacterial activity might be caused by insolubility and no diffusion of silicone materials in agar medium.

Our findings relating to polydimethylsiloxane materials are in agreement with previous studies.[17,19,22,29] *Willerhausen* et al.,[18] in turn, showed a weak antibacterial effect of GuttaFlow against *P. micros*. According to *Nawal* et al.,[21] however, the nano-silver component added to this material might have acted as a preservative, but it does not contribute towards antimicrobial efficacy. The aforementioned differences may be caused by variations in methodologies.

The agar diffusion test is one of the most often used methods to assess and compare the antibacterial activity of root canal materials.[13,15,17,19] To a certain extent, it helps to imitate a clinical situation since agar, as well as dentine, has a buffering effect.[26] Its main disadvantage is the inability to distinguish between the bactericidal and the bacteriostatic properties of the materials. In addition, the size of the inhibition zones not only depends on the antimicrobial agent content in the materials, but also on their solubility and diffusibility in an agar medium.[19] Although the environment of root canals is dissimilar to the agar, bacteria can colonize the dentinal tubules, thus requiring the materials to have a high diffusion capability to be an effective antimicrobial agent.[25]

Conlusions

The present findings indicated that the antimicrobial activity of root canal sealers varied considerably depending on the type of material and the tested bacterial species. Most of the sealers demonstrated antibacterial action on *Fusobacterium nucleatum, Porphyromonas gingivalis*, and *Peptostreptococcus anaerobius.*

References

1. *Siqueira Jr JF, Rocas IN*: Clinical implication and microbiology of bacterial persistence after treatment procedures. J Endod 2008; 34: 1291-1301.
2. *Richardson R, Mordan N J, Figueiredo JAP, Ng Y-L, Gulabivala*: Microflora in teeth associated with apical periodontitis: a methodological observational study comparing two protocols and three microscopy techniques. Int Endod J 2009; 42: 908-921.
3. *Bogen G, Slots J*: Black-pigmented anaerobic rods in closed periapical lesions. Int Endod J 1999; 32: 204-210.
4. *Cao H, Qi Z, Jiang H, Zhao J, Liu Z, Tang Z*: Detection of *Porphyromonas endodontalis, Porphyromonas gingivalis* and *Prevotella intermedia* in primary endodontic infections in a Chinese population. Int Endod J 2012; 45: 773-781.
5. *Chavez de Paz Villanueva LE*: *Fusobacterium nucleatum* in endodontic flare-ups. Oral Surg Oral Med Oral Pathol Oral Radiol Oral Endod 2002; 93: 179-183.
6. *Jacinto RC, Gomes BPF, Shah HN, Ferraz CC, Zaia AA, Souza-Filho F*: Incidence and antimicrobial susceptibility of *Porphyromonas gingivalis* isolated from mixed endodontic infections. Int Endod J 2006; 39: 62-70.
7. *Yang Q-B, Fan LN, Shi Q*: Polymerase chain reaction-denaturing gradient gel electrophoresis, cloning, and sequence analysis of bacteria associated with acute periapical abscess in children. J Endod 2010; 36: 218-23.
8. *Yamasaki M, Nakata K, Imalzumi I, Iwama A, Nakane A, Nakamura H*: Cytotoxic effect of endodontic bacteria on periapical fibroblasts. J Endod 1998; 24: 534-539.
9. *Huynh T, Kapur RV, Kaplan CV, Cacalano N, Haake SK, Wenyuan S, et al.*: The role of aggregation in *Fusobacterium nucleatum* – induce immune cell death. J Endod 2011; 37: 1531-1535.
10. *Kaplan CW, Lux R, Kinder Haake S, Shi W*: The *Fusobacterium nucleatum* outer membrane protein RadD is an arginine-inhibitable adhesion required for inter-species adherence and the structured architecture of multispecies biofilm. Mol Microbiol 2009; 71: 35-47.
11. *Sandqvist G, Figdor D*: Life as an endodontic pathogen. Endod Topics 2003; 6: 3-28.
12. *Jacinto RC, Montagner F, Signoretti FGC, Almeida GC, Gomes BPF*: Frequency, microbial interactions, and antimicrobial susceptibility of *Fusobacterium nucleatum* and *Fusobacterium necrophorum* isolated from primary endodontic infections. J Endod 2008; 34: 1451-1456.
13. `*Gjorgievska E, Apostolska S, Dimkov A., Nicholson JW, Kaftandzieva A:* Incorporation of antimicrobial agents can be used to enhance the antibacterial effect of endodontic sealers. Dent Mater 2013; 29: 29-34.
14. *Haapasalo M, Shen J:* Current therapeutic options for endodontic biofilms. Endod Topics 2012; 22: 79-98.
15. *Siqueira Jr JFS, Favieri A, Gahyva SMM, Moraes SR, Lima KC, Lopes HP*: Antimicrobial activity and flow rate of newer and established root canal sealers. J Endod 2000; 26: 274-277.
16. *Siqueira Jr JFS, Goncalves RB*: Antibacterial activities of root canal sealers against selected anaerobic bacteria. J Endod 1996; 22: 79-80.
17. *Heyder M, Kranz S, Volpel A, Pfister W, Watts DC, Jandt KD, et al.*: Antibacterial effect of different sealers on three bacterial species. Dent Mater 2013; 29: 542-549.
18. *Willershausen I, Callaway A, Briseno B, Willershausen B:* In vitro analysis of the cytotoxicity and the antimicrobial effect of four endodontic sealers. Head Face Med 2011; 7-15.
19. *Cobankara FK, Altinoz HC, Erganis O, Kav K, Belli S*: *In vitro* antibacterial activities of root-canal sealers by using two different methods. J Endod 2004; 30: 57-60.
20. *Merdad K, Pascon AE, Kulkarni G, Santerre P, Friedman S*: Short-term cytotoxicity assessment of components of the Epiphany resin-percha obturating system by indirect and direct contact milipore filter assay. J Endod 2007; 33: 24-27.
21. *Nawal RR, Parande M, Sehgal R, Naik A, Rao NR*: A comparative evaluation of antimicrobial efficacy and flow properties for Epiphany, Guttaflow and AH Plus sealer. Int Endod J 2011; 44: 307-313.
22. *Slutzky-Goldberg I, Slutzky H, Solomonov M, Moshonov J, Weiss EI, Matalon S*: Antibacterial properties of four endodontic sealers. J Endod 2008; 34: 735-738.
23. Desai S, Chandler N: Calcium hydroxide-based root canal sealer: a review. J Endod 2009; 35: 475-480.
24. *Pawińska M, Skrzydlewska E*: Release of hydroxyl

ions from calcium hydroxide preparations used in endodontic treatment. Ann Acad Med Bialostocensis 2003; 48: 145-149.

25. *Mickel AK, Nguyen TH, Chogle S*: Antimicrobial activity of endodontic sealers on *Enterococcus faecalis*. J Endod 2003; 29: 257-258.
26. *Ebert J, Roggendorf MJ, Frank K, Petschelt A*: Antimicrobial activity of various "active" gutta-percha points against *Enterococcus faecalis* in simulated root canals. Int Endod J 2008; 41: 249-257.
27. *Ergucu Z, Hiller KA, Schmalz G*: Influence of dentin on the effectiveness of antibacterial agents. J Endod 2007; 3: 124-129.
28. *Haapasalo M, Qian W, Portenier I, Waltimo T*: Effect of dentin on the antimicrobial properties of endodontic medicaments. J Endod 2007; 33: 917-925.
29. *Ozcan E, Eldeniz AU, Ari H*: Bacterial killing by several root filling materials and methods in an ex vivo infected root canal model. Int Endod J 2011; 44: 1102-1109.

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Received: 20th May 2016
Accepted: 14th December 2016