

Efficacy of Bioceramic and Calcium Hydroxide-Based Root Canal Sealers against Pathogenic Endodontic Biofilms: An *In vitro* Study

Abstract

Background: Complete eradication of root canal pathogens cannot be predictably achieved by chemomechanical preparation and root canal disinfection. Therefore, an obturation material that has superior antimicrobial activity and sealing ability is required to inactivate residual microbes and prevent them from reentering the root canal system. Recently developed bioceramic root canal sealers are hydraulic cement which form calcium hydroxide during the hydration process. Like calcium hydroxide sealers, they exert an antimicrobial effect by releasing hydroxyl ions and increasing the pH. **Objective:** The objective of this study was to evaluate and compare the antimicrobial activity of a calcium hydroxide-based sealer and two bioceramic sealers against *Porphyromonas gingivalis*, *Enterococcus faecalis*, and *Candida albicans* biofilms. **Materials and Methods:** The sealers were dissolved in sterile saline to obtain supernatants. Biofilm formation assays, colony counting, and real-time polymerase chain reaction (PCR) were performed to evaluate the antimicrobial activity of each supernatant. The data were analyzed using one-way analysis of variance. **Results:** All sealers exerted effects against all three microbial biofilms. The biofilm formation assays showed that the bioceramic sealers were more effective against *P. gingivalis* and *E. faecalis* biofilms. In contrast, colony counting and real-time PCR showed that the calcium hydroxide sealer was significantly more effective than the bioceramic sealers. All tests showed that the calcium hydroxide sealer was more effective against *C. albicans*, with the colony count and real-time PCR results showing statistically significant differences. **Conclusion:** The calcium hydroxide-based sealer was more effective than the bioceramic sealers in eradicating pathogenic root canal biofilms.

Keywords: Antimicrobial activity, bioceramic sealer, calcium hydroxide-based root canal sealer, *Candida albicans*, *Enterococcus faecalis*, *Porphyromonas gingivalis*

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Dear Dr Santoso,

An edited and formatted version of your manuscript ccd_198_21 entitled "Efficacy of bioceramic and calcium hydroxide-based root canal sealers against pathogenic endodontic biofilms: an in vitro study" which is scheduled for publication in a forthcoming issue of Contemporary Clinical Dentistry, has been uploaded on our site: <https://review.jow.medknow.com/ccd>.

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List of corrections

Page number	Column (Left / Right)	Paragraph number from top	Line number from top of paragraph	Delete this text (Error)	Replace deleted text with (correction)
2 onwards					Running title: Efficacy of Bioceramic and Calcium Hydroxide Sealers against Endodontic Biofilms
1	Left		0		Conform article type: Original Article
1	Left		3		Conform title:

					“Efficacy of Bioceramic and Calcium Hydroxide–Based Root Canal Sealers Against Pathogenic Endodontic Biofilms: An <i>In Vitro</i> Research”
1	Right		12	¹ <i>Department of Conservative Dentistry, Faculty of Dentistry, Trisakti University, West Jakarta, Java, Indonesia,</i>	¹ <i>Department of Conservative Dentistry, Faculty of Dentistry, Universitas Trisakti, Jakarta, Indonesia,</i>
1	Right		15	² <i>???, Postgraduate Program of Conservative Dentistry, Faculty of Dentistry, Trisakti University, West Jakarta, Java, Indonesia</i>	² <i>Conservative Dentistry Postgraduate Program, Department of Conservative Dentistry, Faculty of Dentistry, Universitas Trisakti, Jakarta, Indonesia</i>
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1	Right		34	Revised: ???	Revised: 16-Jun-2021
1	Right		39	Address for correspondence: <i>Dr. Jessica Santoso, Departemen Konservasi Gigi, Fakultas Kedokteran Gigi, Universitas Trisakti, Kampus B – Jl. Kyai Tapa 260, Grogol, Jakarta Barat 11440, Jakarta, Java, Indonesia. E-mail: drg.jessica.santoso@gmail.com</i>	Address for correspondence: <i>Dr. Jessica Santoso, Department of Conservative Dentistry, Faculty of Dentistry, Universitas Trisakti, Campus B – Jl. Kyai Tapa 260, Grogol, Jakarta Barat 11440, Jakarta, Indonesia. E-mail: drg.jessica.santoso@gmail.com</i>
3	Right	3	36	One-way ANOVA test was performed, followed by Tukey’s honestly significant difference <i>post hoc</i> test <i>were used</i> to determine the	One-way ANOVA test was performed, followed by Tukey’s honestly significant difference <i>post hoc</i> test to determine the significance of

				significance of the differences between experimental groups.	the differences between experimental groups.
3	Right	3	41	The statistical analysis was performed using IBM SPSS® Statistics version 25 (IBM, USA).	The statistical analysis was performed using IBM® SPSS® Statistics 25.0 Desktop for Windows (IBM Corporation, New York, USA).
7	Right		15	13. Šimundić Munitić M, Poklepović Peričić T, Utrobičić A, Bago I, Puljak L. Antimicrobial efficacy of commercially available endodontic bioceramic root canal sealers: A systematic review. PLoS One 2019;14:e0223575	13. Šimundić Munitić M, Poklepović Peričić T, Utrobičić A, Bago I, Puljak L. Antimicrobial efficacy of commercially available endodontic bioceramic root canal sealers: A systematic review. PLoS One 2019;14(10):1-20.
7	Right		35	19. American Association of Endodontists. Guide to Clinical Endodontics. 6th ed. ???; ???; ???. Available from: https://www.aae.org/specialty/clinical-resources/guide-clinical-endodontics . [Last accessed on 2019 Nov 11].	19. American Association of Endodontists. Guide to Clinical Endodontics. 6th ed. Chicago: American Association of Endodontists; 2013. Available from: https://www.aae.org/specialty/clinical-resources/guide-clinical-endodontics . [Last accessed on 2019 Nov 11].
8	Left		19	34. Trott A. Wound cleansing and irrigation. In: Wounds and Laceration. 4th ed., Ch. 7. ???; Elsevier Saunders; 2012. p. 73-4.	34. Trott A. Wounds and Laceration. 4th ed. Philadelphia: Elsevier Saunders; 2012. Chapter 7, Wound cleansing and irrigation. p. 73-4.
8	Left		25	36. Miglani R, Shankar, Indira R, Ramachandran S. An <i>in vitro</i> evaluation of calcium hydroxide root canal sealers and its effect on six microorganisms. J Conserv Dent 2007;10:99-103.	36. Miglani R, Shankar, Indira R, Ramachandran S. An <i>in vitro</i> evaluation of calcium hydroxide root canal sealers and its effect on six microorganisms. J Conserv Dent 2007;10:99-103.

					(Note: the original article and citation doesn't provide the author's first name or initial)
8	Left		44	41. Rezende GC, Massunari L, Queiroz IO, Gomes Filho JE, Jacinto RC, Lodi CS, <i>et al.</i> Antimicrobial action of calcium hydroxide-based endodontic sealers after setting, against <i>E. faecalis</i> biofilm. Braz Oral Res 2016;30 :???.	41. Rezende GC, Massunari L, Queiroz IO, Gomes Filho JE, Jacinto RC, Lodi CS, <i>et al.</i> Antimicrobial action of calcium hydroxide-based endodontic sealers after setting, against <i>E. faecalis</i> biofilm. Braz Oral Res 2016;30(1):1-6.
8	Right		8	46. Weckwerth PH, Lima FL, Greatti VR, Duarte MA, Vivan RR. Effects of the association of antifungal drugs on the antimicrobial action of endodontic sealers. Braz Oral Res 2015;29 :???.	46. Weckwerth PH, Lima FL, Greatti VR, Duarte MA, Vivan RR. Effects of the association of antifungal drugs on the antimicrobial action of endodontic sealers. Braz Oral Res 2015;29(1) :1-7.
8	Right		14	48. Eldesouky HE, Mayhoub A, Hazbun TR, Seleem MN. Reversal of azole resistance in <i>Candida albicans</i> by sulfa antibacterial drugs. Antimicrob Agents Chemother 2018;62 :???.	48. Eldesouky HE, Mayhoub A, Hazbun TR, Seleem MN. Reversal of azole resistance in <i>Candida albicans</i> by sulfa antibacterial drugs. Antimicrob Agents Chemother 2018;62(3):1-12.
8	Right		22	51. Marek C, Timmons S. Antimicrobials in pediatric dentistry. In: Nowak A, editor. Pediatric Dentistry: Infancy through Adolescence. 6th ed. ??? : Elsevier; 2019. p. 128-41.	51. Marek C, Timmons S. Antimicrobials in pediatric dentistry. In: Nowak A, editor. Pediatric Dentistry: Infancy through Adolescence. 6th ed. Philadelphia: Elsevier; 2019. p. 128-41.
8	Right		35	55. Hoshino RA, Silva GF, Delfino MM, Guerreiro-Tanomaru JM, Tanomaru-Filho M, Sasso-Cerri E, <i>et</i>	55. Hoshino RA, Silva GF, Delfino MM, Guerreiro-Tanomaru JM, Tanomaru-Filho M, Sasso-Cerri E, <i>et al.</i> Physical

				a/. Physical properties, antimicrobial activity and <i>in vivo</i> tissue response to apexit plus. Materials (Basel) 2020;13 :???.	properties, antimicrobial activity and <i>in vivo</i> tissue response to apexit plus. Materials (Basel) 2020;13(5):1-17.

Reply to the reviewers' comments

Reviewer Number	Original comments of the reviewer	Reply by the author(s)	Changes done on page number and line number
	<ul style="list-style-type: none"> • In title – mention as in-vitro study 		No changes
	<ul style="list-style-type: none"> • Brain heart infusion broth normally helps in proliferation of bacteria. Hence buffered phosphate buffer is preferred rather than BHI 	Brain heart infusion broth was used in this study as the culture media, in accordance with previous studies. Buffered phosphate buffer or PBS was used to wash microtiter plate before crystal violet assay.	No changes
	<ul style="list-style-type: none"> • This type of evaluation does not simulate an <i>in vivo</i> or clinical situation because the oral cavity bacteria are presented in a biofilm form, which guarantee a protected mode of growth in a hostile environment. Further there are other factors as pH modulation capabilities of dentin. Pure laboratory studies when compared with dentin model design have shown contradictory results. 	Pure laboratory studies were done to examine the ability of the sealers to eradicate oral pathogens which were often found inside the root canal and periapical tissue; without being affected by root canal anatomy, biomechanical preparation, root canal irrigation and disinfection, etc.	Page 12, Line 10

	So clinical translational is questionable. How would you justify your study?	Further clinical studies should be done to examine and compare the sealers' antimicrobial effect in human teeth <i>in vitro</i> , or <i>in vivo</i> if possible, as hostile environment inside the root canal along with other factors/variables may affect the final result differently.	
	<ul style="list-style-type: none"> • Provide reference for Pathogen culture procedures 	Pathogen culture procedures were based on the Clinical and Laboratory Standards Institute.	Page 5, Line 3. Reference 32.
	<ul style="list-style-type: none"> • References are not as per journal instructions- journal abbreviation, delete issue numbers recheck references 59, 36, 18, 19 	All issue numbers deleted.	Page 14-18: References no 19, 18, 37, 60 along with all journals containing issue numbers

Efficacy of Bioceramic and Calcium Hydroxide-Based Root Canal Sealers against Pathogenic Endodontic Biofilms: An *In vitro* Study

Abstract

Background: Complete eradication of root canal pathogens cannot be predictably achieved by chemomechanical preparation and root canal disinfection. Therefore, an obturation material that has superior antimicrobial activity and sealing ability is required to inactivate residual microbes and prevent them from reentering the root canal system. Recently developed bioceramic root canal sealers are hydraulic cement which form calcium hydroxide during the hydration process. Like calcium hydroxide sealers, they exert an antimicrobial effect by releasing hydroxyl ions and increasing the pH. **Objective:** The objective of this study was to evaluate and compare the antimicrobial activity of a calcium hydroxide-based sealer and two bioceramic sealers against *Porphyromonas gingivalis*, *Enterococcus faecalis*, and *Candida albicans* biofilms. **Materials and Methods:** The sealers were dissolved in sterile saline to obtain supernatants. Biofilm formation assays, colony counting, and real-time polymerase chain reaction (PCR) were performed to evaluate the antimicrobial activity of each supernatant. The data were analyzed using one-way analysis of variance. **Results:** All sealers exerted effects against all three microbial biofilms. The biofilm formation assays showed that the bioceramic sealers were more effective against *P. gingivalis* and *E. faecalis* biofilms. In contrast, colony counting and real-time PCR showed that the calcium hydroxide sealer was significantly more effective than the bioceramic sealers. All tests showed that the calcium hydroxide sealer was more effective against *C. albicans*, with the colony count and real-time PCR results showing statistically significant differences. **Conclusion:** The calcium hydroxide-based sealer was more effective than the bioceramic sealers in eradicating pathogenic root canal biofilms.

Keywords: Antimicrobial activity, bioceramic sealer, calcium hydroxide-based root canal sealer, *Candida albicans*, *Enterococcus faecalis*, *Porphyromonas gingivalis*

Introduction

Microorganisms and microbial products are the main etiologic factors associated with pulp disease and periapical lesions.^[1] Gram-negative anaerobic bacterial species, one of which is *Porphyromonas gingivalis*, are often found in primary infections with necrotic pulp.^[2-4] In secondary infections or apical periodontitis lesions in teeth that have undergone endodontic treatment, *Enterococcus faecalis* is the most frequently detected bacterium,^[5-7] while *Candida albicans* is the most common fungal species.^[3,6]

Bacterial infections in the root canal may cause periapical and pulp inflammation and lead to failure of a previous root canal treatment.^[8] Even well-performed endodontic treatments may fail to completely eradicate persistent bacteria

that cannot be reached by instruments or are resistant to disinfection procedures.^[6] Microbes in persistent infection cases, such as *E. faecalis* and *C. albicans*, can invade and colonize dentin, live in conditions of nutrient deficiency, and resist calcium hydroxide treatments.^[9-11]

Root canal treatments are performed to eliminate biofilms, eradicate infections, and prevent microorganisms from infecting or reinfesting root canals and periradicular tissue^[5,12] by filling and sealing the root canal spaces.^[13] However, complex root canal anatomical variations, such as isthmuses and canal ramifications, are often undetected, making the complete elimination of root canal bacteria uncertain.^[14,15] Therefore, root canal filling materials should have the ability to eradicate biofilms and residual bacteria after instrumentation and root canal irrigation.^[16-18]

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Root canal sealers are used in conjunction with biologically acceptable solid or semisolid obturating materials to achieve adequate sealing of the root canal system.^[19] Sealers with excellent sealing ability and antibacterial activity are required to control endodontic infections, inhibit harboring residual bacterial growth, prevent nutrient leakage and root canal reinfection, and facilitate the healing process of apical and periapical tissues.^[8,16,17]

Calcium hydroxide-based sealers have antimicrobial properties^[20,21] and osteogenic-cementogenic potential.^[20,22] Calcium hydroxide exerts an antibacterial effect by releasing hydroxyl ions and increasing pH levels.^[23,24] Previous studies have shown that calcium hydroxide root canal sealers have a wide range of antibacterial effects and lower cytotoxicity than other sealers. Their disadvantage, however, is that they dissolve more easily, forming gaps inside the root canal,^[25] and thus do not meet Grossman's criteria for an ideal root canal sealer.^[21]

In recent years, bioceramic materials have been developed as root canal sealers. These materials are calcium silicate-based cement with the addition of several oxide components.^[20] They are known to have bioactive properties that can stimulate tissue repair and induce mineralization and are therefore considered suitable for root canal sealing applications.^[8,26]

Bioceramic sealers are also advantageous because they are biocompatible, bioactive, nontoxic, presented an alkaline pH, and dimensionally stable with minimal expansion.^[27,28] The two main features of these materials are their hydraulic nature and their reactivity due to the formation of calcium hydroxide that is leached in a solution.^[26] Their hydrophilic properties mean that they are not sensitive to moisture and blood contamination, which makes them ideal for the treatment of root canals and tubules, which are naturally moist.^[29] After setting, they become hard and insoluble, providing excellent long-term sealing.^[30] Moreover, they provide pH values above 12 due to a hydration reaction whereby calcium hydroxide is formed and breaks down into calcium and hydroxyl ions.^[30]

Although several *in vitro* studies have reported varying degrees of antimicrobial activity of bioceramic sealers, safe conclusions cannot be drawn because of the high heterogeneity that characterize these studies.^[13] Like calcium hydroxide sealers, bioceramic sealers exert an antimicrobial effect by releasing hydroxyl ions and increasing the pH.^[31] However, only a few studies have investigated the effects of bioceramic sealers against *P. gingivalis*, *E. faecalis*, and *C. albicans*. Therefore, this study aimed to examine the differences in the ability of two bioceramic sealers and a calcium hydroxide-based sealer to eradicate *P. gingivalis*, *E. faecalis*, and *C. albicans* biofilms.

Materials and Methods

Sample preparation and study design

A laboratory experimental study with a posttest-only control design was conducted to investigate the efficacy of root canal sealers against endodontic biofilms. The root canal sealers tested were BioRoot™ RCS (Septodont, France), Sure-Seal Root™ (Sure Dent, South Korea), and Sealapex™ (Kerr, USA). Table 1 shows the chemical composition and characteristics of the sealers. Each sealer was prepared according to its manufacturer's instructions, distributed to three silicone molds with a diameter of 7 mm and a depth of 3 mm, and incubated at 37°C under humid conditions for 24 h. After setting, the sealer blocks were powdered using a mortar and pestle and then dissolved in a sterile saline solution (Otsu NS NaCl 0.9%; Otsuka, Indonesia) to obtain suspensions in concentrations of 50 mg/mL. Each suspension was homogenized for 10 min and then centrifuged at 4000×g at 25°C for 10 min to obtain a supernatant. The supernatants were then filtered with 0.22-µm filters (Minisart® single filter; Sartorius, Germany) to remove any deposits.

Pathogen cultures

Quantities of 50 µL of *P. gingivalis* (ATCC® 33277™) and *E. faecalis* (ATCC® 29212™) bacterial suspensions were cultivated aerobically in 1.9 mL of brain–heart infusion (BHI) broth (Sigma-Aldrich, USA). A total of 50 µL of *C. albicans* (ATCC® 10231™) suspension was cultivated in 1.9 mL of Sabouraud dextrose broth (Sigma-Aldrich, USA). All suspensions were homogenized using a vortex mixer (MX-S; DLAB Scientific, PRC) and then incubated at 37°C for 24 h. The cultures were diluted to an equivalent of optical density (OD)₆₀₀ 0.132 (McFarland 0.5 or 1.5 × 10⁸ CFU/mL) in accordance with the inoculum density standards of the Clinical and Laboratory Standards Institute.^[32]

Biofilm formation assay

Quantities of 200 µL of suspensions were inoculated in 96-well microplates (Biologix, USA) and incubated again under anaerobic conditions at 37°C for 24 h to form biofilms. The supernatants of bacteria and fungi that had been incubated were discarded until only the biofilms at the bottoms of the well plates remained. Subsequently, the supernatants of the three sealers were distributed 200 µL per well, repeated six times for each experimental group, and then incubated at 37°C for 24 h.

After incubation for 24 h, four out of six wells containing biofilms and sealer supernatants of each experimental group were rinsed with 200 µL of phosphate-buffered saline (PBS; VWR Life Science, USA). Suspensions from the remaining wells were transferred into microtubes for colony counts and real-time quantitative polymerase chain reaction (qPCR). Biofilm staining was performed

Table 1: Compositions, manufacturers, and lot numbers of the tested sealers

Material	Composition	Producer	Lot number	Notes
BioRoot™ RCS	Powder: Tricalcium silicate, zirconium dioxide, and povidone Liquid: Water, calcium chloride, and polycarboxylate	Septodont, France	B23103	Bioceramic sealer
Sure-Seal Root™	Calcium silicate, calcium aluminate, calcium aluminoferrite, calcium sulfate, radiopacifier, and thickening agent	Sure Dent, South Korea	WR953100	Bioceramic sealer
Sealapex™	Base paste: N-ethyl-o-toluene sulfonamide, calcium oxide, zinc oxide, and zinc distearate Catalyst paste: Methyl salicylate, 2,2-dimethylpropane-1,3-diol, and isobutyl salicylate	Kerr, United States	7081108	Calcium hydroxide-based sealer

RCS: Root canal sealer

with 200 µL of 0.5% crystal violet solution (Merck, USA) in each well for 15 min and then rinsed again with PBS. A total of 200 µL of absolute ethanol (EMSURE®; Merck, USA) was inserted into each well, and absorption measurements were conducted using a microplate reader (MP96; Safas, Monaco) at a wavelength of 595 nm.

Counting of microbial colony-forming units

Aliquots of 100 µL of each treatment were pipetted to perform two serial 100-fold dilutions. A total of 2 µL of the diluted suspension was plated on a sterile BHI agar medium (Oxoid, USA). The suspensions in all Petri dishes (Iwaki Glass, Indonesia) were incubated at 37°C for 24 h (anaerobically for the *P. gingivalis* and *E. faecalis* suspensions). The number of bacterial and fungal colonies formed was observed, calculated, and converted to colony-forming units per milliliter.

Real-time quantitative polymerase chain reaction

Bacterial and *Candida* DNA extraction was performed using the heat-shock method. The suspensions were centrifuged at 4500×g for 15 min. The supernatants formed were then discarded to get a pellet filled with pathogens. The pellets were resuspended with 100 µL of ddH₂O and then homogenized for 5 min. Microtubes were heated in a dry block thermostat (Bio TDB-100; Biosan, Latvia) at 100°C for 20 min and then immediately placed in an ice bath for 10 min. After the extraction, the samples were homogenized again with a vortex mixer. Centrifugation was performed again at 10,000×g for 2 min. The supernatants containing DNA were transferred into new microtubes and stored at 4°C. The samples were evaluated after 24 h.

Mixtures of 20 µL were prepared for the qPCR test, each containing 2 µL of DNA, 10 µL of qPCR Mix (HOT FIREPol® SolisGreen qPCR Mix; Solis BioDyne, Estonia), 6 µL of nuclease-free water, 1 µL of forward primer, and 1 µL of reverse primer. The primers used were AGGCAGCTTGCCATACTGCG (forward) and ACTGTTAGCAACTACCGATGT (reverse) for *P. gingivalis* with an amplicon length of 127 bp, 5'-GTT TAT GCC GCA TGG CATAAG AG-3' (forward) and 5'-CCG TCA GGG GAC GTT CAG-3' (reverse) for *E. faecalis* with an amplicon length of 310 bp, and CCC AGT

CTT TCA CAA GCA GTA AAT (forward) and GTA AAT GAG TCA TCA ACA GAA GCC (reverse) for *C. albicans* with an amplicon length of 356 bp.

The mixtures were homogenized and distributed to 48-well PCR plates (Biologix, USA). *P. gingivalis*, *E. faecalis*, and *C. albicans* were identified by PCR amplification of the 16S rRNA gene. Real-time PCR was performed using a thermal cycler (Applied Biosystems, StepOne Real-Time PCR System™; Thermo Fisher Scientific, USA) with SYBR® Green I fluorophore. The program, temperature, and plate design were set on a computer connected to the thermocycler. In each well, the gene expression intensity was measured and the threshold cycle (Ct) values, that is, the relative values representing the number of cycles in which the amplified DNA reaches a threshold level, were obtained. The Ct values were then converted to colony-forming units per milliliter using the standard curve of each microbe.

Statistical analysis

The data obtained from the biofilm formation assays, colony counts, and real-time PCR, all ratio scale data, were tested for normality using the Shapiro–Wilk test. One-way ANOVA test was performed, followed by Tukey's honestly significant difference *post hoc* test were used to determine the significance of the differences between experimental groups. The level of statistical significance was set to $P < 0.05$. The statistical analysis was performed using IBM SPSS® Statistics version 25 (IBM, USA).

Results

Porphyromonas gingivalis biofilms

The results of the biofilm formation assays showed that the BioRoot RCS bioceramic sealer was the most effective in eradicating *P. gingivalis* biofilms (OD: 0.155), followed by the Sure-Seal Root bioceramic sealer and the Sealapex calcium hydroxide-based sealer. However, the colony count results [Figure 1] showed that Sealapex was the most effective against *P. gingivalis* (7.5×10^6 CFU/mL), followed by BioRoot RCS and Sure-Seal Root. The difference was statistically significant ($P < 0.05$). Real-time PCR also showed that Sealapex was significantly more

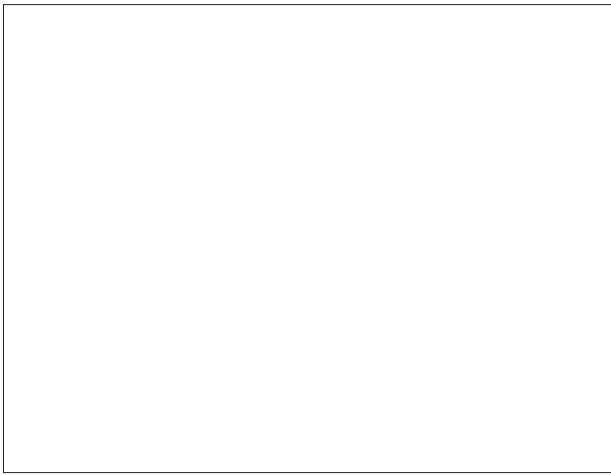


Figure 1: Colony counts of *Porphyromonas gingivalis* on brain–heart infusion agar plates with treatments in duplicate

effective (2.345×10^4 CFU/mL) than both bioceramic sealers ($P < 0.01$). Figure 2 shows the results of the activity of the three root canal sealers against *P. gingivalis* biofilms and the statistically significant differences between the groups.

Enterococcus faecalis biofilms

The biofilm formation assays showed that Sure-Seal Root was the most effective in eradicating *E. faecalis* biofilms (OD: 0.181), followed by BioRoot RCS and Sealapex. The antibacterial effect of both bioceramic sealers was significantly stronger than that of Sealapex ($P < 0.01$). However, qPCR showed that Sealapex was the most effective against *E. faecalis* (1.38×10^5 CFU/mL), followed by Sure-Seal Root and BioRoot RCS. Moreover, the colony count results showed that Sealapex was highly effective against *E. faecalis*, with 0 CFU/mL formed [Figure 3]. In both tests, the antibacterial effect of Sealapex was significantly stronger than that of BioRoot RCS ($P < 0.05$). Although Sealapex has better antibacterial effect, it was not statistically significant when compared to Sure-Seal Root. The results of the antimicrobial activity measurements of three root canal sealers against *E. faecalis* biofilms and the statistically significant differences between the groups are shown in Figure 4.

Candida albicans biofilms

The biofilm formation assays showed that Sealapex was the most effective in eradicating *C. albicans* biofilms (OD: 0.45), followed by BioRoot RCS and Sure-Seal Root. However, the differences between the sealers were not statistically significant. The colony count [Figure 5] and qPCR results also showed that Sealapex was the most effective (0 CFU/mL and 496.172 CFU/mL, respectively). In both tests, the antimicrobial effect of Sealapex was significantly stronger than that of Sure-Seal Root ($P < 0.05$). Sealapex also performed better compared to BioRoot RCS, although it was not statistically significant. Figure 6 shows

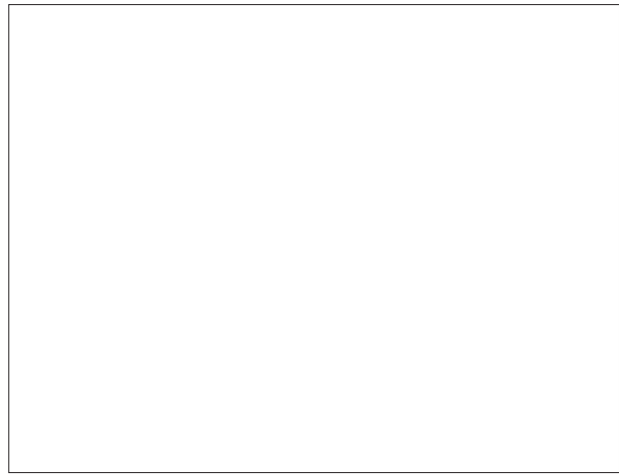


Figure 2: Antimicrobial activity measurement results of the root canal sealers against *Porphyromonas gingivalis*. (a) Biofilm formation assay; (b) Colony counts; (c) Real-time polymerase chain reaction. The error bars indicate standard deviations of the means. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

the results of the antimicrobial activity measurements of the three root canal sealers against *C. albicans* biofilms and the statistically significant differences between the groups.

Discussion

Both bioceramic and calcium hydroxide-based sealers are able to inhibit bacterial growth at the concentration of 50 mg/mL in concordance with prior studies.^[8,17,31] The pH measurement done for each sealer supernatant showed a value of 11.55 for the BioRoot RCS suspension, 11.64 for Sure-Seal Root, and 12.47 for Sealapex. An alkaline pH causes denaturation of cytoplasmic membrane proteins, lipid peroxidation, and inhibition of DNA replication and acts as a physical barrier that restricts microbial growth.^[33]

The biofilm formation assays showed that the bioceramic BioRoot RCS sealer was the most effective against *P. gingivalis*, followed by the bioceramic Sure-Seal Root sealer and the calcium hydroxide-based Sealapex sealer. These findings are comparable with the results of a previous study using biofilm assays that reported that calcium silicate-based sealers have a strong antimicrobial effect against Gram-positive *E. faecalis* along with Gram-negative *P. gingivalis* and *Porphyromonas endodontalis* bacteria.^[5]

Antibacterial activity of BioRoot RCS against *P. gingivalis* is due to its tricalcium silicate, povidone, and zirconium oxide contents. When in contact with a liquid, tricalcium silicate reacts and produces hydroxyl ions, which increase the pH in the root canal system^[26] and eradicate Gram-negative bacteria such as *P. gingivalis* by damaging their cell membranes, inhibiting their lipopolysaccharides, and denaturing their proteins.^[23,24] Povidone does not have microbicidal properties but slows the release of excess ions, thus maintaining long-term antimicrobial activity.^[34] Zirconium oxide damages bacterial membranes and prevents further growth.^[35]

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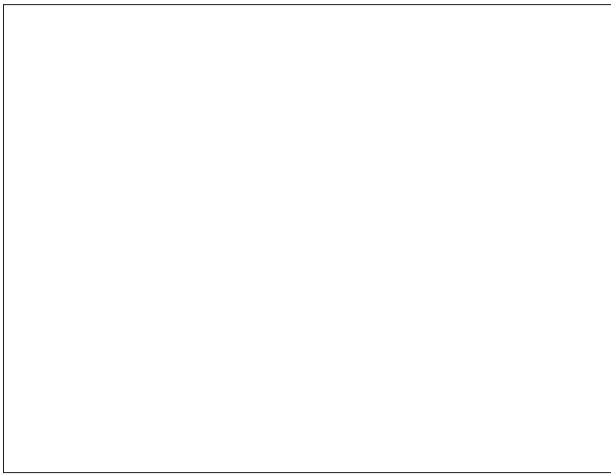


Figure 3: Colony counts of *Enterococcus faecalis* on brain–heart infusion agar plates with treatments in duplicate

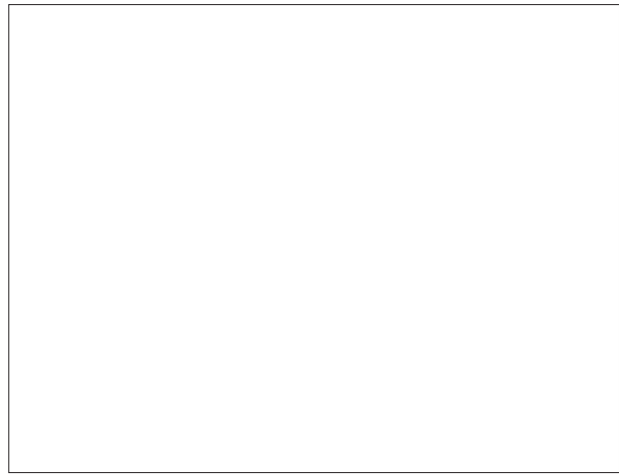


Figure 4: Antimicrobial activity measurement results of the root canal sealers against *Enterococcus faecalis*. (a) Biofilm formation assay; (b) Colony counts; (c) Real-time polymerase chain reaction. The error bars indicate standard deviations of the means. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$



Figure 5: Colony counts of *Candida albicans* on brain–heart infusion agar plates with treatments in duplicate

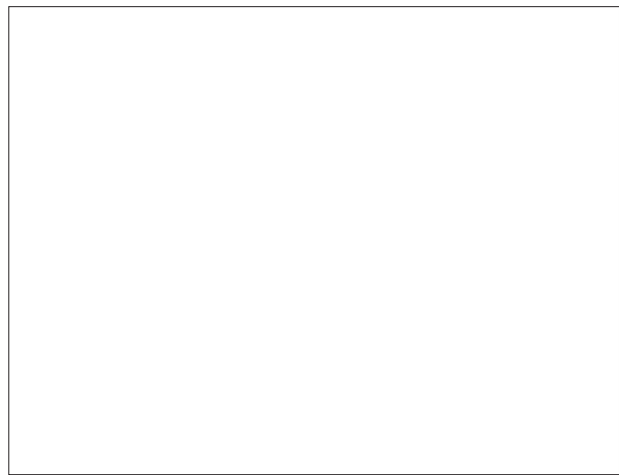


Figure 6: Antimicrobial activity measurement results of the root canal sealers against *Candida albicans*. (a) Biofilm formation assay; (b) Colony counts; (c) Real-time polymerase chain reaction. The error bars indicate standard deviations of the means. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

The results of colony count and real-time PCR (qPCR) showed that Sealapex was the most effective against *P. gingivalis*, followed by the two bioceramic sealers. Previous studies revealed that the antibacterial activity of calcium hydroxide depends on the total concentration and release rate of hydroxyl ions.^[36] Calcium hydroxide-based sealers can break down into calcium ions and hydroxyl ions, causing an increase in pH up to 12.5, which can damage the microbial cytoplasmic membrane.^[37] This high pH is obtained within 1 h and can last for 30 days.^[21] Hydroxyl ions are highly oxidant free radicals that are highly reactive to cytoplasmic membrane biomolecules, thus compromising the integrity of the cytoplasmic membrane of bacteria and inhibiting the lipopolysaccharides of Gram-negative bacteria.^[38] Moreover, when reacting with carbon dioxide, calcium ions can block the source of respiration of anaerobic bacteria.^[36]

Sure-Seal Root was the most effective against *E. faecalis* bacteria, followed by BioRoot RCS and Sealapex in the biofilm formation assays. A previous study comparing

traditional and bioceramic sealers found that calcium silicate-based sealer (Endoseal MTA; Maruchi, South Korea) was more effective against *E. faecalis* than other sealers, including calcium hydroxide-based sealers, due to the oxidizing components of calcium silicate-based sealers, which exert strong activity against both Gram-positive and Gram-negative bacteria.^[8] The effect of Sure-Seal Root against *E. faecalis* is due to its calcium silicate, calcium aluminate, and calcium sulfate contents. Calcium silicate is effective against bacteria that are tolerant of alkaline conditions.^[39] The oxide components of bioceramic sealers damage the cell walls of Gram-positive bacteria and increase the permeability of molecules into their cytoplasm.^[40] These components also facilitate the penetration of calcium hydroxide into the cytosol and the denaturation of bacterial DNA and proteins.^[24]

Colony counting and qPCR showed that the calcium hydroxide-based Sealapex was the most effective against

E. faecalis, followed by Sure-Seal Root and BioRoot RCS. These results are in line with previous studies which found that calcium hydroxide-based sealers showed excellent antimicrobial activity after 24, 48, and 72 h and 7 days using agar diffusion tests.^[41,42] Similar results were obtained using direct contact tests, which showed that Sealapex acted against *E. faecalis* within 24 h^[43] and was still more effective than other sealers after 7 days.^[27] This is probably due to the release of hydroxyl ions, which create an unfavorable environment for the growth of microorganisms by increasing the pH.^[41,42] The antimicrobial mechanism of calcium hydroxide-based sealers is influenced by the speed at which they break down into calcium ions and hydroxyl ions.^[44] The decomposition of hydroxyl ions results in a high pH environment, thus inhibiting enzymatic activity, which is important for the metabolism and growth of microbes, as well as cell division.^[21]

The biofilm assays, colony formation counts, and qPCR showed that Sealapex was the most effective against *C. albicans*, followed by BioRoot RCS and Sure-Seal Root. These results are consistent with those of a previous study reporting that calcium hydroxide had stronger effects against *E. faecalis* and *C. albicans* than MTA and Portland cement.^[45] Its activity against *C. albicans* is due to the formation of inhibition zones.^[46,47] Sulfonamides contained in Sealapex may increase its antibacterial and antifungal activity. Sulfa antibacterial agents can inhibit the formation and growth of *C. albicans* biofilms, which are usually more resistant to antifungal agents than planktonic cells,^[48] by preventing the biosynthesis of folic acid.^[49] As eukaryotic microbes such as fungi synthesize folate *de novo*, inhibition of folate biosynthesis causes folate deficiency, inhibiting cell growth.^[50] Sulfa drugs block the folate pathway to dihydropteroate synthase enzyme, which *C. albicans* needs to convert para-aminobenzoic acid to dihydrofolate. The interruption of the folate pathway in *C. albicans* can also inhibit the biosynthesis of ergosterol.^[48] Without ergosterol, which maintains the integrity of the cell membrane, its permeability increases.^[51]

Previous studies had investigated the antibacterial effectiveness of calcium silicate-based root canal sealers with varied results, mostly against *E. faecalis*.^[1,52] Our study examined the antimicrobial activities of both bioceramic and calcium hydroxide-based root canal sealers against *E. faecalis*, and lesser studied root canal pathogens such as *P. gingivalis* and *C. albicans*, which are mostly found in primary and persistent infection, respectively.^[53,54] The three testing methods performed in this study were biofilm formation assays, counting of colonies formed on BHI agar media, and real-time PCR. Similarity of the results obtained by different methods would determine the quality and validity of the conclusion.

However, a shortcoming in our study is that the biofilm formation assays produced contradictory results with

the colony counts and qPCR regarding the sealers' effectiveness against *P. gingivalis* and *E. faecalis*. This discrepancy may have been caused by deposits produced by the sealer supernatants, which implies that filtering with 0.22- μ m filters did not ensure the supernatants were free from deposits. In previous studies, most deposits found in Sealapex and BioRoot RCS were due to calcium precipitation.^[55,56] As biofilm formation assay measures biomass by absorption of crystal violet stains,^[57] this may lead to readings of both precipitation and biofilm mass formed at the bottom of the well plate, causing a higher OD. This finding, however, was not seen in the *C. albicans* experimental groups, although it should be noted that in the biofilm formation assay, there were no significant differences between the three tested sealers. Future studies should take care to obtain deposit-free supernatants, therefore increasing the accuracy of biofilm formation assay results.

Molecular methods for microbial identification, such as qPCR, have advantages over culture methods. Real-time PCR can identify microbes more accurately, does not require microbial cultures, and thus can detect both cultivable and noncultivable species, and can be performed quickly on many samples.^[58] Culture methods, on the other hand, can only detect microbes that can be cultivated and form colonies.^[57,58] Differences in the detection methods may sometimes cause discrepancies between the results of qPCR and those of cultivation methods.^[57] Previous studies indicate that such discrepancies in both methods may be explained by the inability of cultivation methods to distinguish between close related bacteria, the different threshold levels of both methods, and the problems of keeping pathogenic bacteria viable, which is required for standard cultivation.^[59] In this study, however, colony counting and qPCR produced mostly consistent results. In this study, however, the results of colony counting and qPCR are mostly consistent. The possible explanations to these findings are the use of standard reference strain (ATCC) instead of microbial isolates and that the pathogens used in this study are facultative anaerobes, which are easier to cultivate and kept viable.

Within the limitations of this study, most tests showed that Sealapex was the most effective against all pathogens, namely *P. gingivalis*, *E. faecalis*, and *C. albicans*. However, as antimicrobial properties are only one of the many properties required for an ideal root canal sealer, other properties of bioceramic sealers, such as dimensionally stable and insoluble in tissue fluids,^[16] could make this type of sealer worth considering as a root canal obturation materials.^[20,60] Therefore, further *ex vivo* studies should be done to examine and compare the antimicrobial effect of the root canal sealers on extracted human teeth which will be shaped, cleaned, and obturated using the tested sealers, as environment inside the root canal along with other factors and variables may affect the final result differently. Further

studies should also investigate the increase or decrease in the antimicrobial effects of each sealer over time, as both bioceramic and calcium hydroxide-based root canal sealers' antimicrobial effects were based on the release of hydroxyl ions and increase pH levels that were obtained over time. Moreover, similar studies could examine the sealers' effects against other root canal pathogens.

Conclusion

All three root canal sealers had antimicrobial effects. Real-time PCR showed that the calcium hydroxide-based sealer was more effective than the bioceramic sealers against *P. gingivalis* biofilms. Both colony counts and qPCR showed that the calcium hydroxide-based sealer was also more effective against *E. faecalis*. Furthermore, all three tests performed showed that it was also the most effective against *C. albicans* biofilms. These results suggest that calcium hydroxide-based sealer was the most effective against all pathogenic root canal biofilms studied.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

- Jafari F, Kafil HS, Jafari S, Aghazadeh M, Momeni T. Antibacterial activity of MTA Fillapex and AH 26 root canal sealers at different time intervals. *Iran Endod J* 2016;11:192-7.
- Baumgartner JC, Siqueira JF, Sedgley CM, Kishen A. Microbiology of endodontic disease. In: Ingle JI, Bakland LK, Baumgartner JC, editors. *Ingle's Endodontics*. 6th ed. Ontario: People's Medical Publishing House; 2008. p. 221-308.
- Siqueira JF, Rôças IN, Ricucci D. Microbial and nonmicrobial etiologies of endodontic diseases. In: Rotstein I, Ingle J, editors. *Ingle's Endodontic*. 7th ed. North Carolina, USA: PMPH; 2019. p. 85-102.
- Rôças IN, Siqueira JF, Andrade AF, Uzeda M. Identification of selected putative oral pathogens in primary root canal infections associated with symptoms. *Anaerobe* 2002;8:200-8.
- Siqueira JF, Rôças IN. Microbiology of endodontic infections. In: Hargreaves KM, Berman LH, Rotstein I, editors. *Cohen's Pathways of the Pulp*. 11th ed. St. Louis: Elsevier; 2016. p. 599-629.
- Siqueira JF, Rôças IN. Microbiology of apical periodontitis. In: Ørstavik D, editor. *Essential Endodontology: Prevention and Treatment of Apical Periodontitis*. 3rd ed. New Jersey: Wiley-Blackwell; 2020. p. 91-127.
- Chavez de Paz L. Gram-positive organisms in endodontic infections. *Endod Top* 2004;9:79-96.
- Shin JH, Lee DY, Lee SH. Comparison of antimicrobial activity of traditional and new developed root sealers against pathogens related root canal. *J Dent Sci* 2018;13:54-9.
- Siqueira JF, editor. *Treatment of Endodontic Infections*. 1st ed. London: Quintessence; 2011. p. 1-40, 95-153, 311-40.
- Waltimo TM, Ørstavik D, Sirén EK, Haapasalo MP. *In vitro* susceptibility of *Candida albicans* to four disinfectants and their combinations. *Int Endod J* 1999;32:421-9.
- Haapasalo M, Udnaes T, Endal U. Persistent, recurrent, and acquired infection of the root canal system post-treatment. *Endod Top* 2003;6:29-56.
- Peters OA, Koka RS. Preparation of coronal and radicular spaces. In: Ingle JI, Bakland LK, Baumgartner JC, editors. *Ingle's Endodontics*. 6th ed. Ontario: People's Medical Publishing House; 2008. p. 877-991.
- Šimundić Munitić M, Poklepović Peričić T, Utrobičić A, Bago I, Puljak L. Antimicrobial efficacy of commercially available endodontic bioceramic root canal sealers: A systematic review. *PLoS One* 2019;14:e0223575.
- Gutmann JL, Fan B. Tooth morphology, isolation, and access. In: Hargreaves KM, Berman LH, Rotstein I, editors. *Cohen's Pathways of the Pulp*. 11th ed. St. Louis: Elsevier; 2016. p. 130-208.
- Ørstavik D. Endodontic treatment of apical periodontitis. In: Ørstavik D, editor. *Essential Endodontology: Prevention and Treatment of Apical Periodontitis*. 3rd ed. New Jersey: Wiley-Blackwell; 2020. p. 314.
- Kapralos V, Koutroulis A, Ørstavik D, Sunde PT, Rukke HV. Antibacterial activity of endodontic sealers against planktonic bacteria and bacteria in biofilms. *J Endod* 2018;44:149-54.
- Poggio C, Trovati F, Ceci M, Colombo M, Pietrocola G. Antibacterial activity of different root canal sealers against *Enterococcus faecalis*. *J Clin Exp Dent* 2017;9:e743-8.
- Spangberg LS, Haapasalo M. Rationale and efficacy of root canal medicaments and root filling materials with emphasis on treatment outcome. *Endod Top* 2002;2:35-58.
- American Association of Endodontists. *Guide to Clinical Endodontics*. 6th ed. ???, ???, ??? Available from: <https://www.aae.org/specialty/clinical/resources/guide-clinical-endodontics>. [Last accessed on 2019 Nov 11].
- Johnson WT, Kulild JC. Obturation of the cleaned and shaped root canal system. In: Hargreaves KM, Berman LH, Rotstein I, editors. *Cohen's Pathways of the Pulp*. 11th ed. St. Louis: Elsevier; 2016. p. 349-88.
- Desai S, Chandler N. Calcium hydroxide-based root canal sealers: A review. *J Endod* 2009;35:475-80.
- Johnson JD. Root canal filling materials. In: Ingle JI, Bakland LK, Baumgartner JC, editors. *Ingle's Endodontics*. 6th ed. Ontario: People's Medical Publishing House; 2008. p. 1019-52.
- Mohammadi Z, Shalavi S, Yazdizadeh M. Antimicrobial activity of calcium hydroxide in endodontics: A review. *Chonnam Med J* 2012;48:133-40.
- Siqueira JF Jr, Lopes HP. Mechanisms of antimicrobial activity of calcium hydroxide: A critical review. *Int Endod J* 1999;32:361-9.
- Ersahan S, Aydin C. Solubility and apical sealing characteristics of a new calcium silicate-based root canal sealer in comparison to calcium hydroxide-, methacrylate resin- and epoxy resin-based sealers. *Acta Odontol Scand* 2013;71:857-62.
- Camilleri J. Will bioceramics be the future root canal filling materials? *Curr Oral Health Rep* 2017;4:228-38.

- 1 27. Zhang H, Shen Y, Ruse ND, Haapasalo M. Antibacterial activity
2 of endodontic sealers by modified direct contact test against
3 *Enterococcus faecalis*. J Endod 2009;35:1051-5.
- 4 28. Wang Z. Bioceramic materials in endodontics. Endod Top
5 2015;32:3-30.
- 6 29. Trope M, Bunes A, Debelian G. Root filling materials and
7 techniques: Bioceramics a new hope? Endod Top 2015;32:86-96.
- 8 30. Debelian G, Trope M. The use of premixed bioceramic materials
9 in endodontics. G Ital Endod 2016;30:70-80.
- 10 31. Morgental R, Vier-Pelisser F, Oliviera S, Antunes F, Cogo D,
11 Kopper P. Antibacterial activity of two MTA-based root canal
12 sealers. Int Endod J 2011;44:1128-33.
- 13 32. Clinical and Laboratory Standards Institute (CLSI). Methods
14 for Dilution Antimicrobial Susceptibility Tests for Bacteria that
15 Grow Aerobically; Approved Standard. 10th ed. Pennsylvania:
16 Clinical and Laboratory Standards Institute; 2015. p. 17-20.
- 17 33. Buch A, Asthana G, Parmar G. An *in vitro* comparison of pH
18 changes of roots following canal dressing with calcium hydroxide
19 points, conventional calcium hydroxide paste and a commercial
20 calcium hydroxide paste. Endodontology 2012;24:12-9.
- 21 34. ~~Trott A. Wound cleansing and irrigation. In: Wounds and
22 Laceration. 4th ed., Ch. 7. ??? Elsevier Saunders; 2012. p. 73-4.~~
- 23 35. Guerreiro-Tanomaru JM, Trindade-Junior A, Costa BC,
24 da Silva GF, Drullis Cifali L, Basso Bernardi MI, et al. Effect of
25 zirconium oxide and zinc oxide nanoparticles on physicochemical
26 properties and antibiofilm activity of a calcium silicate-based
27 material. ScientificWorldJournal 2014;2014:975213.
- 28 36. Miglani R, Shankar, Indira R, Ramachandran S. An *in vitro*
29 evaluation of calcium hydroxide root canal sealers and its effect
30 on six microorganisms. J Conserv Dent 2007;10:99-103.
- 31 37. Ramachandra PK, Krishnegowda SC, Jaganath BM, Rudranik S,
32 Manjula CG, Kurup NB, Madanan S. *In vitro* comparative
33 evaluation of the antibacterial and antifungal activities of
34 different root canal sealers against endodontic pathogens. Int J
35 Prev Clin Dent Res 2016;3:261-6.
- 36 38. Chowdury R, Alam S, Rubby G, Rabbi G. Antimicrobial
37 efficacy of endomethasone and sealapex sealers against specific
38 endodontic pathogens. City Dent Coll J 2013;10:27-30.
- 39 39. Lim ES, Park YB, Kwon YS, Shon WJ, Lee KW, Min KS.
40 Physical properties and biocompatibility of an injectable
41 calcium-silicate-based root canal sealer: *In vitro* and *in vivo*
42 study. BMC Oral Health 2015;15:129.
- 43 40. Hajipour MJ, Fromm KM, Ashkarran AA,
44 Jimenez de Aberasturi D, de Larramendi IR, Rojo T, et al.
45 Antibacterial properties of nanoparticles. Trends Biotechnol
46 2012;30:499-511.
- 47 41. ~~Rezende GC, Massunari L, Queiroz IO, Gomes Filho JE,
48 Jacinto RC, Lodi CS, et al. Antimicrobial action of calcium
49 hydroxide-based endodontic sealers after setting, against
50 *E. faecalis* biofilm. Braz Oral Res 2016;30:???.~~
- 51 42. Dalmia S, Gaikwad A, Samuel R, Aher G, Gulve M, Kolhe S.
52 Antimicrobial efficacy of different endodontic sealers against
53 *Enterococcus faecalis*: An *in vitro* study. J Int Soc Prev
54 Community Dent 2018;8:104-9.
- 55 43. Heyder M, Kranz S, Völpel A, Pfister W, Watts DC, Jandt KD,
56 et al. Antibacterial effect of different root canal sealers on three
bacterial species. Dent Mater 2013;29:542-9.
44. Estrela C, Mamede Neto I, Lopes HP, Estrela CR,
Pécora JD. Root canal filling with calcium hydroxide using
different techniques. Braz Dent J 2002;13:53-6.
45. Basir L, Khanehmajedi M, Khosravi A, Ansarifard S.
Investigating the antimicrobial activity of different root canal
filling pastes in deciduous teeth. Clin Cosmet Investig Dent
2019;11:321-6.
46. ~~Weekwerth PH, Lima FL, Greatti VR, Duarte MA, Vivan RR.
Effects of the association of antifungal drugs on the antimicrobial
action of endodontic sealers. Braz Oral Res 2015;29:???.~~
47. 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;38:539-43.
48. ~~Eldesouky HE, Mayhoub A, Hazbun TR, Seleem MN. Reversal
of azole resistance in *Candida albicans* by sulfa antibacterial
drugs. Antimicrob Agents Chemother 2018;62:???.~~
49. Bush K, Freudenberger JS, Slusarchyk DS, Sykes RB, Meyers E.
Activity of sulfa drugs and dihydrofolate reductase inhibitors
against *Candida albicans*. Experientia 1982;38:436-7.
50. Castelli LA, Nguyen NP, Macreadie IG. Sulfa drug screening
in yeast: Fifteen sulfa drugs compete with p-aminobenzoate in
Saccharomyces cerevisiae. FEMS Microbiol Lett 2001;199:181-4.
51. ~~Marek C, Timmons S. Antimicrobials in pediatric dentistry.
In: Nowak A, editor. Pediatric Dentistry: Infancy through
Adolescence. 6th ed. ??? Elsevier; 2019. p. 128-41.~~
52. Singh G, Gupta I, Elshamy FM, Boreak N, Homeida HE.
In vitro comparison of antibacterial properties of
bioceramic-based sealer, resin-based sealer and zinc oxide
eugenol based sealer and two mineral trioxide aggregates. Eur
J Dent 2016;10:366-9.
53. Gomes BP, Pedrosa JA, Jacinto RC, Vianna ME, Ferraz CC,
Zaia AA, et al. *In vitro* evaluation of the antimicrobial activity of
five root canal sealers. Braz Dent J 2004;15:30-5.
54. Gomes BP, Pinheiro ET, Gadê-Neto CR, Sousa EL, Ferraz CC,
Zaia AA, et al. Microbiological examination of infected dental
root canals. Oral Microbiol Immunol 2004;19:71-6.
55. ~~Hoshino RA, Silva GF, Delfino MM, Guerreiro-Tanomaru JM,
Tanomaru-Filho M, Sasso-Cerri E, et al. Physical properties,
antimicrobial activity and *in vivo* tissue response to apexit plus.
Materials (Basel) 2020;13:???.~~
56. Prüllage RK, Urban K, Schäfer E, Dammaschke T. Material
properties of a tricalcium silicate-containing, a mineral trioxide
aggregate-containing, and an epoxy resin-based root canal sealer.
J Endod 2016;42:1784-8.
57. Azeredo J, Azevedo NF, Briandet R, Cerca N, Coenye T,
Costa AR, et al. Critical review on biofilm methods. Crit Rev
Microbiol 2017;43:313-51.
58. Siqueira JF, Rôças IN. Exploiting molecular methods to explore
endodontic infections: Part I – Current molecular technologies
for microbiological diagnosis. J Endod 2005;31:411-23.
59. Jervøe-Storm PM, Koltzsch M, Falk W, Dörfler A, Jepsen S.
Comparison of culture and real-time PCR for detection
and quantification of five putative periodontopathogenic
bacteria in subgingival plaque samples. J Clin Periodontol
2005;32:778-83.
60. Kulild JC, Karabucak B. Obturation. In: Torabinejad M,
Walton RE, Fouad AF, editors. Endodontics: Principles and
Practice. 5th ed. St. Louis: Elsevier Saunders; 2015. p. 316-37.

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