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Antibacterial effectiveness of calcium silicate-based root canal sealers - A systematic review and meta-analysis

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Abstract

It is highly essential that the root canal sealers used for obturation possess sufficient antibacterial action to act against the residual micro bes in the root canal system. This review aims to evaluate and compare the anti-bacterial effectiveness of calcium silicate-based root canal sealers in comparison with epoxy resin-based root canal sealers.

This systematic review and meta-analysis is registered in the International Prospective Register of Systematic Reviews PROSPERO (registration number: CRD 42021239328). Literature search was carried out in Medline through various electronic databases, websites and hand searched in journals and open grey records from January 2010 to January 2022.Invitro studies com paring antibacterial action of calcium silicate and epoxy resin sealers on Entero coccus faecalis by direct contact test were considered. After thorough analysis, 10 studies were considered for qualitative analysis and 4 were taken up for quantitative analysis. Meta-analysis, using random-effects model, was applied with Rev Man 5.3, for 24–48-hour contact period of Enterococcus faecalis with epoxy resin and calcium silicate root canal sealers. No statistically significant difference between the two groups, although epoxy resin-based sealers show better action in 24-48 hours, with a standardized mean difference of 3.55 (95% CI = -1.54 to 8.64; p=0.17). Taking in the potential limitations, epoxy resin sealers show better antibacterial property than calcium silicate

sealers, especially in 24-48 hours, although not statistically significant.

Keywords: Antibacterial agent, Calcium silicate, Enterococcus faecalis, Root canal sealer.

Introduction

The ultimate clinical objective of root canal treatment is to achieve a three- dimensional seal or obturation of the endodontic spaces after being completely cleaned, shaped, and disinfected. Although the crucial part is pro per chemo-mechanical preparation,[1] preventing the entry of micro-organisms from the oral flora into the root canal system and periapical tissues is one of the most important principles for a successful treatment outcome.[2] Because contemporary reports have shown that no available filling materials or techniques can produce a complete seal of the entire root canal system, the root filling should at least entomb residual microorganisms, impeding their access to peri radicular tissues.[3] This is accomplished by completely obturating the root canal system.

A common cause for failure of endodontic therapy is micro leak age due to an inadequate apical seal. If this part of the canal is not adequately cleaned and obturated, the residual bacteria may multiply and cause treatment failure due to apical percolation of tissue fluid.[4]

According to the American Association of Endodontists (AAE) root canal sealers are used in conjunction with a bio logically acceptable semi-solid or solid obturating material to establish an adequate seal of the root canal system also known as a hermetic seal.[5] With the increasing understanding of the etiopathogenesis of periapical disease, and inflammatory status of the periapical tissue, modifications have been made to improve the chemical and physical properties of sealers [6] to serve other functions as well, apart from the anti-microbial property.

Among the clinically available root canal sealers, epoxy resin-based sealers are currently widely used. Several studies have considered AH Plus to be the gold standard for sealers,[7,8]

however, it does have its limitations. Calcium and silicate-based dental cement have been introduced to modern dentistry over the past two decades and is well established. [9,10] They are preferred for their bio compatibility, good seal, and excellent flow.[11]

The sealer inside the root canal will be in direct contact with the residual micro-organisms, thus it is imperative to have good antibacterial efficacy.[12-14]

AH 26 has significant antibacterial activity against obligate anaerobes.[15,16] Calcium silicate sealers show suit able anti-bacterial action based on their property of releasing calcium and hydroxyl ions thus maintaining an alkaline ph. Root canal sealers with sufficient anti-bacterial properties are being preferred of late, to reduce the incidence of endodontic failures.[17]

Furthermore, the changes in surface chemistry may affect the anti-microbial properties of the sealers, especially in the deeper portions of the root canal system.[18,19]

Previous literature searches were unable to compare the anti-bacterial properties between these two groups of sealers, specifically by a direct contact test. This outlook will give a clear view on the antibacterial property of the sealers.

Therefore, the objective of this systematic review and meta-analysis is to summarise the outcomes of the in vitro studies on the antimicrobial effectiveness of calcium silicate-based sealers when compared with that of epoxy resin sealers on Enterococcus faecalis (E. Faecalis) based on the results of direct contact test.

Materials and methods

A. Protocol registration and reporting

The present systematic review was registered at the National Institute for Health Research (PROSPERO) International Prospective Register of Systematic Reviews (Registration number: CRD42021239328) and designed according to the PRISMA (Preferred Reporting Items for Systematic Review and Meta - Analyses) guidelines 2020.

B. Research question

The controlled vocabulary (MeSH terms) and free terms were used to define the search strategy based on the elements of the PICOS question:

- Population: E. faecalis strain in cell culture plates
- Intervention: Calcium silicate-based root canal sealers
- Comparison: Epoxy resin sealers
- Outcome: Antimicrobial efficacy of calcium silicate-based root canal sealers
- Study design: In vitro studies

C. Eligibility criteria

Articles were considered eligible if they met the following criteria: (i) Studies with E. Faecalis as one of the bacterial strains, (ii) Antibacterial activity measured by direct contact test, with results obtained as the number of colony forming units / ml (CFU / ml), (iii) Studies with epoxy resin-based sealers as one of the comparators.

Exclusion criteria were

(i) Antibacterial activity measured by any other test.

(ii) Studies carried out by direct contact test with results as a measure of turbidity.

(iii) Studies not in the English language

(iv) Studies unrelated to the topic of interest,

(v) Literature reviews and letters to the editor.

D. Search strategy

A comprehensive search was carried out by two reviewers (M.M, M.W) in Medline through various electronic databases like PubMed, Scopus, Web of Science, Google Scholar, and Embase from January 2010 to January 2022.

The search terms used are summarised in Table 1. Literature search was also carried out on websites and major endodontic journals for in-press articles. Reference sections of all included articles were hand-searched. In addition, a grey literature search was carried out in Open Grey (opengrey.org).

E. Study Selection

Two reviewers (M.M, and M.W) independently scanned the titles of the articles obtained initially. The abstracts of the relevant articles were reviewed. Full text was referred when the information obtained through titles and abstracts was insufficient.

Inclusion was based on the agreement between the two reviewers. In case of a disagreement, a third reviewer (D.D) was consulted.

F. Assessment of risk of bias

The risk of bias assessment was based on a previous systematic review by AL Shwaimi E et al.[20]. The evaluation was based on the description of the para meters, such as the presence of control, description of sample size calculation, the use of materials according to the Manu facturer's instructions, samples prepared by the single operator, the amount of sealer used, time of evaluation of the antimicrobial activity, and blinding of the observer. If the authors reported the para meter, the article had a Y (yes) for that specific para meter; if not, the article received a N (no). Articles reporting 1 to 3 items were classified as high risk of bias, 4 to 5 as medium risk, and 6 to 7 as low risk.

G. Data Extraction

Data were extracted from the final included studies and entered in a data extraction sheet, created using Micro soft Excel spreadsheet. The data was categorized as mentioned in table 2.

H. Meta-analysis

Heterogeneity was noted in the 10 included studies, mainly in terms of sealer setting and time interval for anti-bacterial activity evaluation. Based on the homogeneity of the other parameters, meta-analysis was conducted for four studies, with a duration of direct contact of the sealers with E. faecalis being 24 to 48 hours. Rev Man 5.3 software was used for meta-analysis using the random effects model.

Results

A. Study selection and descriptive analysis

A total of 895 articles were identified from various sources, including 869 articles from electronic database screening, and 13 articles from registers. Websites and citation searching yielded 13 articles. From the initial 882 studies identified through databases and registers, after the removal of duplicates, a total of 317 articles were assessed for eligibility, among which 287 articles were excluded as they were irrelevant. The eligibility criteria were applied to the remaining 30 articles, out of which 22 were excluded due to various reasons. Among the 13 articles identified from websites and citation searching, 11 articles were excluded. Hence, 10 studies that fulfilled the inclusion criteria were included for qualitative analysis, while four of these were included for meta-analysis. Figure 1 shows the PRISMA 2020 flow diagram.

All the 10 in-vitro studies were published between January 2010 to January 2022. The antibacterial property evaluation period ranged from 10 minutes to 168 hours. A description of the 10 included studies is shown in table 2. [21-29]

B. Risk of bias assessment

All included studies were assessed for the risk of bias. Inter reviewer reliability for the risk of bias evaluation was very good according to Cohen kappa statistics (k=0.88). Factors taken into consideration were control, sample size calculation, materials used, single opera tor, amount of sealer, time interval, and blinding of the observer; with none of the 10 studies mentioning the sample size, single operator, and blinding. Six studies (60%) presented a medium risk of bias, while four studies (40%) presented a high risk of bias. Results are described according to the parameters considered in the analysis. The results are summarised in figure 2.

C. Qualitative analysis

Period for antibacterial property evaluation

The interval for evaluating the antibacterial action of the sealers ranged from 6 minutes in 3 studies[24,27,28] to 168 hours in 2 studies. [21,22] At 6 and 15 minutes, 2 studies showed that calcium silicate sealers show better action than epoxy resins [24,27] whereas 1 study favoured epoxy resins.[28] According to Huang et al.[20], at 10 and 30 minutes, AH plus showed better action. The most common evaluation period among the studies was 1 hour and 24 hours. 7 studies evaluated the antibacterial action after 1 hour of contact, with 2 studies [21,22] favouring epoxy resins and 5 studies [15,24,26-28] favouring the calcium silicate group. At the 24 - hour interval, 2 studies favoured AH plus [23,25] and calcium silicate sealers [15,26] each.

However, according to the conclusions of Bose et al.[21] and Candeiro et al.[22], although epoxy resin sealers presented excellent anti-bacterial property initially, after 24 hours, the anti-bacterial property of calcium silicate sealers steadily rose, showing comparable action as that

of the epoxy resins, thus making the results statistic ally non-significant. Epoxy resin sealers showed the acceptable antibacterial property during the initial period of contact after being set, which gradually reduced in the long term. Calcium silicates, on the other hand, although a slow start, showed comparable or even better anti-bacterial property in a longer run as mentioned in the included studies.

Effect of sealer setting on the antibacterial property

The sealers used in the control and test groups were kept in direct contact with the bacterial strain in a freshly mixed state, [15, 21, 23, 25, 26] 20 minutes,[22] 1 day,[29] and 7 days [20, 24,27,28] after mixing. Even though 5 studies had evaluated the antibacterial property of the sealers in the freshly mixed state, only 2 of the studies favoured epoxy resins at all the evaluated contact intervals. [23,25]

According to Bose et al. in 2020,[21] when used in the freshly mixed state, AH plus showed the better antibacterial property at 1-hour interval, but with longer duration of contact, as in 168 hours, Bio root RCS and Total fill BC sealers showed the lowest mean CFU/ml. Candeiro et al. in 2016[22] concluded that when a 20-minute set sealer was used, AH plus was superior with complete E. faecalis elimination throughout the evaluation period of 1 to 168 hours, with Endo sequence BC exhibiting comparable action after 1 hour. In longer periods of sealer setting, as in 1 and 7 days, calcium silicate sealers showed the better anti-bacterial property.[24,27-29]

D. Meta-analysis

The meta-analysis, using the random effects model, was applied with Rev Man 5.3 (Rev Man 5.3, The Nordic Cochrane Centre, Copenhagen). Heterogeneity was assessed by Q test and quantified with I^2 statistics. Four studies were included in the meta-analyses comparing the E. Faecalis colony forming units after 24 to 48 hours of contact time between Calcium silicate-based sealers (Experimental) and Epoxy resin-based sealers (Control). Epoxy resin-based sealers were more effective in terms of reduced CFU/ml after 24 to 48 hours, with a standardized mean difference of 3.55(95% CI= -1.54 to 8.64; Z value=1.37). This difference in CFU/ml after 24 to 48 hours among the two groups was statistically non-significant (p=0.17). The overall heterogeneity between studies was substantial (I^2 = 96 %).

Figure 3 shows the meta-analysis with continuous variable mean difference of colony forming units along with the forest and funnel plot of the studies included.

Discussion

The primary purpose of this systematic review was to summarise the evidence from multiple in vitro studies that have compared the antibacterial efficacy of calcium silicate-based root canal sealers with epoxy resin-based sealers. The 10 studies included tested different calcium silicate sealers with epoxy resin sealers, which are commonly used in dentistry. All the studies mentioned the sealer composition, type of E. faecalis strain and operational information such as the type of test used for evaluating the antibacterial efficacy, amount of sealer, the quantity of bacterial strain taken and their concentration, sealer setting time, and the time interval for evaluating the antibacterial property of the sealer.

Much of the presently published endodontic research is done in vitro. Despite being placed low in the evidence pyramid, results from in vitro studies can provide useful information, and should always be carefully and critically analysed.[30]

The results obtained from in vitro studies are essential in terms of bacterial activity and prevention of reinfection which are better evaluated in vitro without any con

founding factors. More evidence from case-control and cohort studies and even randomised controlled trials are needed to increase the probability that the results obtained by in vitro research do correctly reflect the real events.[31]

E. faecalis, a gram-positive facultative anaerobe, has been reported to be responsible for 90% of the reinfections, persistent infections, and post-endodontic treatment pain.[32] Sedgley et al. in 2006 [17] detected that the prevalence of E. faecalis was significantly higher in retreatment cases (89.6%) than in primary infection (67.5%).

F. Although various studies have evaluated the anti-bacterial efficacy of sealers using Candida and Strep to coccus, E. faecalis forms an integral part due to its significant role in endodontic infections. Molecular studies have shown the presence of several other taxa in root - filled teeth with persistent or secondary in fections.[33] Since E. faecalis is one of the most cultured bacteria in persistent infection cases, most of the studies available direct themselves against this group of bacteria.

Epoxy resin-based sealers are polymeric material, whose anti-microbial activity was thought to be due to formaldehyde release, according to Spans berg et al.[34] whereas Heil et al. stated that it was due to the release of bisphenol A di glycidyl ether.[32] Currently, this group of sealers is replacing the zinc oxide eugenol-based sealers as the new “gold standard”.[7] Due to their sealing ability and bio compatibility, calcium silicate-based root canal sealers are now taking a stride in endodontics. Substantive long-term antimicrobial effectiveness may be due to calcium ion release and the ability to sustain a high pH.[35] Hydrophilicity and calcium hydroxide diffusion also affect the antimicrobial properties.[33] Although calcium silicate is not the primary constituent of MTA Fill apex, studies with the MTA based sealer have been included in this systematic review, to compare all the commercially available sealers in both the groups in terms of antibacterial property, so that the findings are more accurate. Also, 40% of MTA Fill apex contains calcium silicate, present in the form of MTA.[36] After one and seven days of setting, the antibacterial effect of epoxy resin sealers was reduced due to a decrease in the formaldehyde released and other antibacterial substances as stated by Spans berg et al. and Heil et al.[20,32,35] and this may be the reason for overall reduced antibacterial activity of epoxy resin sealers in five of the included studies. However, the results of the study by Shakya et al.[15] are contradictory to this. Zhang et al. in 2009 stated that AH plus lost its antibacterial action after 24 hours, whereas bio ceramic sealer was able to kill E. faecalis only after 24 hours.[12,22,37] The presence of water reduced the activity of the Total fill BC Sealer against S. aureus after 24 hours and 7 days.[25] Antibacterial components of root canal sealers can exhibit toxic effects on host tissues because of the lack of selective toxicity against microbes which reduced with time.[27] This may be the reason for the stronger antimicrobial activity of freshly mixed sealers.

Direct contact test (DCT) overcomes the shortcomings of the Agar diffusion test (ADT), which is one of the most used tests to evaluate the antibacterial efficacy of root canal sealers.[12,38,39]DCT is based on counting the number of microbial colonies on agar plates at various time intervals, following the sealer being kept in contact with the bacterial strain. It can also distinguish between the bactericidal and bacteriostatic properties of the tested sealer. Unlike ADT, even insoluble material can be tested.[13] The main advantage is that it simulates the

natural environment, with the sealer coming in direct contact with the dentine.

Eight out of the ten included studies have determined the anti-bacterial activity after one hour, since in 6 and 15 minutes of evaluation, the sealers may not have sufficient time to interact with the E. faecalis.[28]

The periods of evaluation range from 6 minutes to 168 hours in the included studies, thus explaining the long-term effects of the sealers. Since epoxy resin sealers showed a de creased anti-bacterial activity after setting, and calcium silicate sealers, because of their calcium hydroxide release and high pH showed a gradual increase in their antibacterial efficacy,[22] we could notice a comparable or sometimes even better antibacterial action of calcium silicate sealers with that of epoxy resins, in a longer interval of testing. Bose et al. in 2020[21] and Candeiro et al. in 2016[22] evaluated the results after 168 hours of contact, in which after 24 hours, there was no significant difference between the two groups of sealers, in their antibacterial activity. The amount of sealer which was kept in contact with the bacterial suspension showed heterogeneity, which emphasises the need for more standardised methods.

A. Limitations and future perspectives

Due to heterogeneity in the data, only four studies were included in the meta-analysis. This is because of a lack of uniformity in the methodologies, different evaluation times, quantity of the sealers, sealer setting used in the studies and blinding. Most of the studies were conducted on planktonic bacteria which is not the case intraorally. The effect of dentine on the antimicrobial activity of sealers should also be investigated. There is a need for more randomised controlled trials to determine the antibacterial efficacy of the current sealers in a clinical scenario. Although clinical trials provide the most reliable results, well-designed in vitro studies with high method logical quality could provide beneficial evidence for a clinical situation.

Conclusion

Within the confines of this systematic review and meta-analysis, it can be concluded that no significant difference exists in the antibacterial efficacy of epoxy resin and calcium silicate-based sealers in terms of reduced CFU/ ml of E. faecalis, although epoxy resin sealers showed a slightly better action during the 24 to 48-hour interval.

The anti-bacterial efficacy of epoxy resin-based sealer was highest in the freshly mixed state. The search for root canal sealers with long-lasting antibacterial activity is required in addition to other properties like reduced solubility, good sealing, and bio com partibility, so that an almost bacteria-free environment can be maintained in the root canal space.

References

1. Siqueira Jr JF, Rocas IN, Favieri A, Abad EC, Castro AJ, Gahyva SM. Bacterial leakage in coronally unsealed root canals obturated with 3 different techni ques. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2000; 90 (5):647-50.

2. Muliyar S, Shameem KA, Than Kachan RP, Francis PG, Jaya palan CS, Hafiz KA. Microleakage in endo dontics. J Int Oral Health 2014;6(6):99-104.

3. Sritharan A. Discuss that the coronal seal is more important than the apical seal for endo dontic success. Aust Endod 2002;28(3):112-115.

4. Tajo Nar RG, Sanchez-Mendieta KP, Martínez-Martínez RE, Domínguez-Pérez RA. Periapical healing of endo dontically treated teeth filled only in the apical third: a randomized controlled trial. Eur Endod J 2017; 3 (1): 24-30

5. Dahlkemper PE, Ang DB, Goldberg RA, Rubin RL, Schultz GB, Sheridan BA, et al. Guide to Clinical Endo dontics Sixth Edition. Available at: https://dental.id/wp-content/uploads/2016/08/aae_guidetoclinicalendodontics 6.pdf 2016.

6. Wuersching SN, Diegritz C, Hickel R, Huth KC, Koll muss M. A comprehensive in vitro comparison of the bio logical and physicochemical properties of bio active root canal sealers. Clin Oral Investig. 2022; 26 (10): 6209-6222.

7. Komabayashi T, Colmenar D, Cvach N, Bhat A, Primus C, Imai Y. Comprehensive review of current endodontic sealers. Dent Mater J 2020;9(5):703-720.

8. Silva EJ, Cardoso ML, Rodrigues JP, De-Deus G, Fidalgo TK. Solubility of bio ceramic- and epoxy resin -based root canal sealers: A systematic review and meta-analysis. Aust Endod J 2021;47(3):699-701.

9. Lim M, Jung C, Shin DH, Cho YB, Song M. Calcium silicate-based root canal sealers: a literature review. Restor Dent Endod2020;45(3): e35.

10. Prati C, Gandolfi MG. Calcium silicate bioactive cements: Bio logical per spectives and clinical app lications. Dent Mater 2015;31(4):351-370.

11. Donner Meyer D, Bürklein S, Dammaschke T, Schäfer E. Endo dontic sealers based on calcium silicates: a systematic review. Odonto logy. 2019; 107 (4): 421-436.

12. Zhang H, Shen Y, Ruse ND, Haapasalo M. Antibacterial activity of endodontic sealers by modified direct contact test against Enterococcus faecalis. J Endod 2009;35(7):1051-1055.

13. Baer J, Maki JS. In vitro evaluation of the antimicrobial effect of three endodontic sealers mixed with amoxicillin. J Endod 2010;36(7):1170-3.

14. Huang Y, Li X, Mandal P, Wu Y, Liu L, Gui H, et al. The in vitro antimicrobial activities of four endodontic sealers. BMC Oral Health 2019;19(1):118.

15. Shakya VK, Gupta P, Tikku AP, Pathak AK, Chandra A, Yadav RK, et al. An Invitro Evaluation of Antimicrobial Efficacy and Flow Characteristics for AH Plus, MTA Fill apex, CRCS and Gutta Flow 2 Root Canal Sealer. J Clin Diagn Res 2016;10(8):104-108.

16. Kaur A, Shah N, Log ani A, Mishra N. Biotoxicity of commonly used root canal sealers: A meta-analysis. J Conserv Dent 2015;18(2):83-88.

17. Chopra V, Davis G, Bays an A. Clinical and Radiographic Outcome of Non-Surgical Endodontic Treatment Using Calcium Silicate-Based Versus Resin-Based Sealers—A Systematic Review and Meta-Analys is of Clinical Studies. J Funct Bio mater 2022; 13 (2): 38.

18. Sedgley C, Nagel A, Dahlen G, Reit C, MO lander A. Real-time quantitative polymerase chain reaction and culture analyses of Enterococcus faecalis in root canals. J Endod 2006;32(3):173-177.

19. Dos Santos DC, da Silva Barboza A, Schneider LR, Cuevas-Suárez CE, Ribeiro JS, Damian MF, et al. Anti micro bial and physical properties of experimental endo dontic sealers containing vegetable extracts. Sci Rep 2021;11(1):6450.

20. Al Shwaimi E, Bogari D, Ajaj R, Al-Shahrani S, Almas K, Majeed A. In Vitro Antimicrobial Effective ness of Root Canal Sealers against Enter ococcus faecal is: A Systematic Review. J Endod 2016; 42 (11): 1588-1597.

21. Bose R, Ioannidis K, Foschi F, Bakhsh A, Kelly RD, Deb S, et al. Anti-microbial Effectiveness of Calcium Silicate Sealers against a Nutrient-Stressed Multi species Biofilm. J Clin Med 2020;9(9):2722

22. Candeiro GTM, Moura-Netto C, D'Almeida-Couto RS, Azambuja-Júnior N, Marques MM, Cai S, et al. Cytotoxicity, genotoxicity and antibacterial effectiveness of a bio ceramic endodontic sealer. Int Endod J 2016;49(9):858-864.

23. Ehsani M, Adibi A, Moosavi E, Dehghani A, Khafri S, Adibi E. Antimicrobial activity of three different endo dontic sealers on the enterococcus faecalis and lacto bacillus (in vitro). Caspian J Dent Res 2013;2(2):8-14.

24. Colombo M, Poggio C, Dagna A, Meravini MV, Riva P, Trovati F, et al. Biological and physico-chemical properties of new root canal sealers. J Clin Exp Dent 2018;10(2):120-126.

25. 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(1):149-154

26. Chakraborty T, Taneja S, Malik S. An Anti-micro bial Activity Assessment of Three Endo dontic Sealers on Enter ococcus faecalis, Candida albicans, and Staphyl ococcus aureus by a Direct Contact Test: An In Vitro Study. Int J Sci Stud 2020;8(4):35-39.

27. Poggio C, Trovati F, Ceci M, Colombo M, Pietro cola G. Antibacterial activity of different root canal sealers against Enterococcus faecalis. J Clin Exp Dent 2017; 9 (6):743-748.

28. Hashem Inia M, Razavi An H, Mosleh H, Shakerian B. In vitro evaluation of the antibacterial activity of five sealers used in root canal therapy. Dent Res J (Isfahan) 2017;14(1):62-67.

29. Zordan-Bronzel CL, Tanomaru-Filho M, Rodrigues EM, Chávez-Andrade GM, Faria G, Guerreiro-Tano maru JM. Cytocompatibility, bioactive potential and anti-micro bial activity of an experimental calcium silicate-based endodontic sealer. Int Endod J 2019; 52 (7): 979-986.

30. Roth KA, Friedman S, Lévesque CM, Basrani BR, Finer Y. Microbial biofilm proliferation within sealer-root dentin interfaces is affected by sealer type and aging period. J Endod 2012;38(9):1253-1256.

31. Haapasalo M. Level of evidence in endodontics: what does it mean? Endodontic Topics 2016;34(1):30-41.

32. Heil J, Reifferscheid G, Waldmann P, Leyhausen G, Geurtsen W. Genotoxicity of dental materials. Mutat Res 1996;368(3-4):181-194.

33. Cai M, Abbott P, Castro Salgado J. Hydroxyl Ion Diffusion through Radicular Dentine When Calcium Hy droxide Is Used under Different Conditions. Materials (Basel) 2018;11(1):152.

34. Sprang berg LS, Barbosa SV, Lavigne GD. AH 26 releases formaldehyde. J Endod. 1993;19(12):596-598.

35. Koutroulis A, Kuehne SA, Cooper PR, Camilleri J. The role of calcium ion release on biocompatibility and anti-microbial properties of hydraulic cements. Sci Rep 2019; 9 (1):19019.

36. Abu Zeid S, Edrees HY, Mokeem Saleh AA, Alothmani OS. Physico chemical Properties of Two Generations of MTA-Based Root Canal Sealers. Materials 2021;14(20):5911.

37. Kangarlou A, Neshandar R, Matini N, Dianat O. Antibacterial efficacy of AH Plus and AH26 sealers mixed with amoxicillin, triple antibiotic paste and nano silver. J Dent Res Dent Clin Dent Prospects 2016; 10 (4): 220-225.

38. Weiss EI, Shalhav M, Fuss Z. Assessment of antibacterial activity of endodontic sealers by a direct contact test. Endod Dent Traumatol 1996;12(4):179-184.

39. Çobankara FK, Altinöz HC, Ergani O, Kav K, Belli S. In vitro antibacterial activities of root-canal sealers by using two different methods. J Endod 2004;30(1):57-60.

Legend Table and Figure

Table 1: Search strategy in PubMed database

Sn.	Category	Keywords
1	Population	"Microbiology"[Mesh] OR "Enterococcus faecalis"[Mesh]
2	Intervention	"Root Canal Filling Materials"[Mesh] OR “Root canal therapy” [Mesh] OR "Silicate Cement"[Mesh] OR “Calcium silicate” [Title/abstract]
3	Comparison	"Cytotoxicity, Immuno logic" [Mesh] OR "Epoxy Resins" [Mesh] OR Cytotoxicity * [Title/ abstract] OR “AH Plus” [Title/abstract] OR Sealer*[Title/abstract]
4	Outcome	"Anti-Bacterial"[Mesh] OR "Therapeutic Equivalency"[Mesh] OR "Colony Count, Micro bial"[Mesh] OR Antibacterial*[Title/abstract] OR “Direct contact test” [Title/abstract]
5		1 AND 2 AND 3 AND 4

Table 2: Description of the studies.

Sn.	Author (Year)	Control group sealers	Test group sealers	Sealer amount	Sealer setting	E. faecalis* strain	Bacterial suspension amount	Antibacterial activity evaluation	Summary of study
1.	Bose et al [21] (2020 – United Kingdom)	AH Plus	Bio root RCS, Total fill BC	200 µL	Freshly mixed	OMGS 3202	500µL (3×10^8 CFU/ ml) †	1, 24, 168 Hours	On measuring the antibacterial activity after 1 hour of contact, AH Plus showed better action. After 24 hours and 168 hours of contact, no significant difference between the three groups was seen.
2.	Candeiro et al [22] (2016 - Brazil)	AH plus	Endo sequence BC	180mg for 6 wells	20 minutes	ATCC 29212	500µL (3×10^8 CFU/ ml)	1, 24, 72, 168 hours	AH plus showed significant (complete) inhibition during all the evaluation time periods. Endo sequence BC showed comparable antibacterial effectiveness only after 24 hours. Significant differences between the groups observed only in 1 hour analysis.
3.	Ehsani et al [23] (2013 - Iran)	AH 26	MTA Fill apex	0.1 cc	Freshly mixed	PTCC 1394	0.01 cc (1.5×10^8 CFU/ml)	24 Hours	The highest anti-bacterial activity was noted in the AH 26 group, followed by MTA fill apex for the given duration of contact.

4.	Columbo et al [24] (2018 - Italy)	AH plus, Easy seal	Bio root RCS, Total fill BC, MTA Fill apaex	1 mL suspension	7 days	ATCC 29212	1mL suspension (1.5×10^8 CFU/ml)	6, 15, 60 minutes	Following 6 minutes of contact, Bio root RCS and Total fill BC showed better anti-bacterial action. After 15 and 60 minutes, significant increase in the antibacterial activity in all the tested sealers noted. For every contact time considered, Total fill BC killed almost all the E. faecalis.
5.	Kapralos et al [25] (2018 - Greece)	AH plus	Total fill BC	20µL	Freshly mixed	ATCC 19434	20 µL	24 hours	AH plus showed better antibacterial efficacy than Total fill BC, in the freshly mixed state of the epoxy resin sealer.
6.	Chakraborty et al [26] (2020 - India)	AH plus	MTA fill apex, Endo sequence BC	50 mg sealer for 9 cell culture tubes.	Freshly mixed	ATCC 29212	50 µL (1.5×10^8 CFU/ml)	1 and 24 hours	Endo sequence BC showed lowest microbial count followed by MTA Fill apex. Highest microbial count for AH Plus for both the time intervals was noted.
7.	Shakya et al [15] (2016 - India)	AH plus	MTA Fill apaex.	50mg for 10 wells	Freshly mixed	ATCC 29212	50 µL (1.5×10^8 CFU/ml)	1 and 24 hours	MTA Fill apex showed a better anti bacterial activity at both the observed time intervals, with a statistically significant difference only after 24 hours of contact.
8.	Poggio et al [27] (2017 - Italy)	AH plus, Easy seal	Bio root RCS, Total fill BC, MTA Fill apaex	1ml suspension	7 days	ATCC 29212	1ml suspension (1.5×10^8 CFU/ml)	6, 15, 60 minutes	For every contact time considered, Total fill BC sealer and Easy seal showed higher bactericidal action and killed all the bacteria. AH plus showed increase in antibacterial action after 15 and 60 minutes.
9.	Hashem Inia et al [28] (2017 - Iran)	AH 26	MTA fill apex	1ml suspension	7 days	PTCC 1393	1ml suspension (1.5×10^8 CFU/ml)	6, 15, 60 minutes	6 minutes: MTA fill apex showed better antibacterial action. 15 and 60 minutes: AH 26 appears to show better efficacy

10.	Zordan Bronzel et al [29] (2019 – Brazil)	AH plus	Total fill BC.	Not mentioned	24 hours	ATCC 29212	10 µL (1×10^8 CFU/ ml)	1 hour and 30 minutes	Total fill BC showed statistically signifi cant anti bacterial activity compared to AH plus.

* E. Faecalis: Enterococcus faecalis

† CFU: Colony forming units.

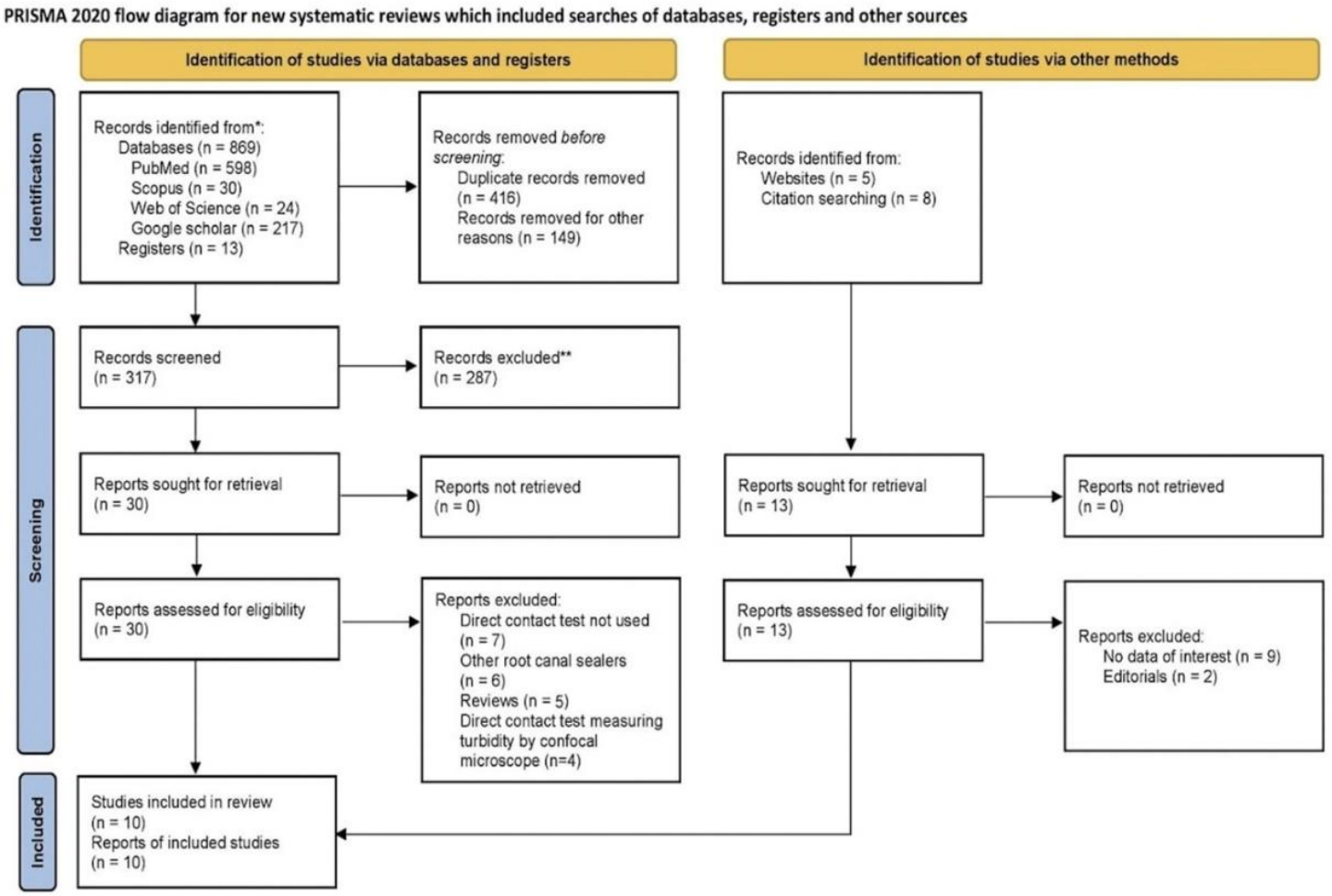
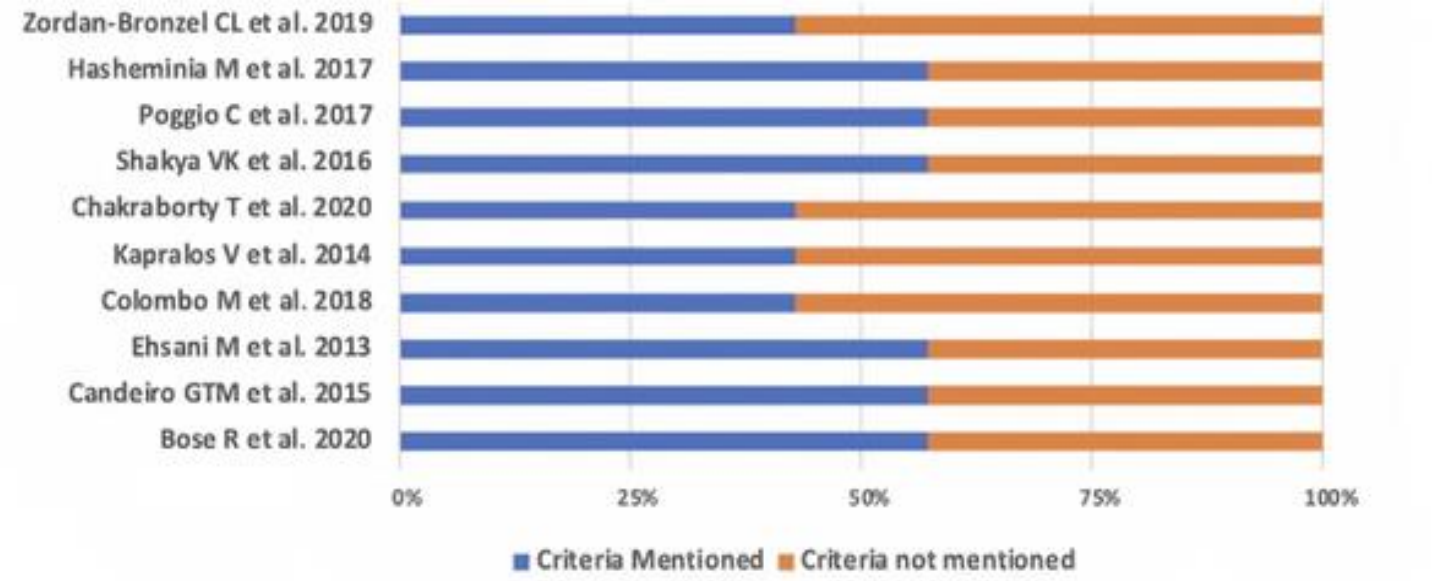


Figure 1: PRISMA (2020) flow diagram for study identification

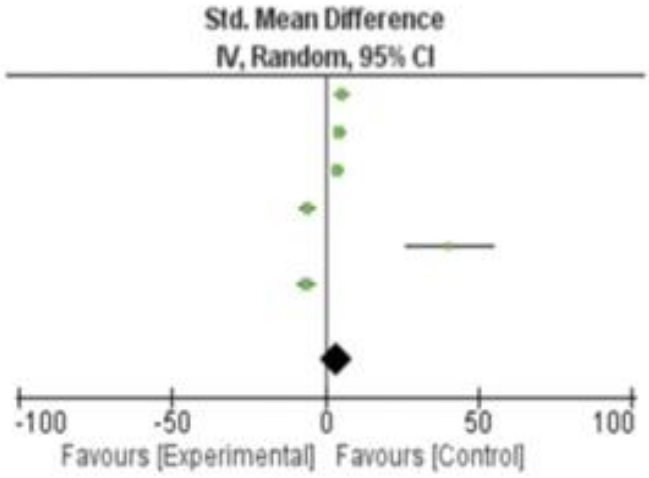


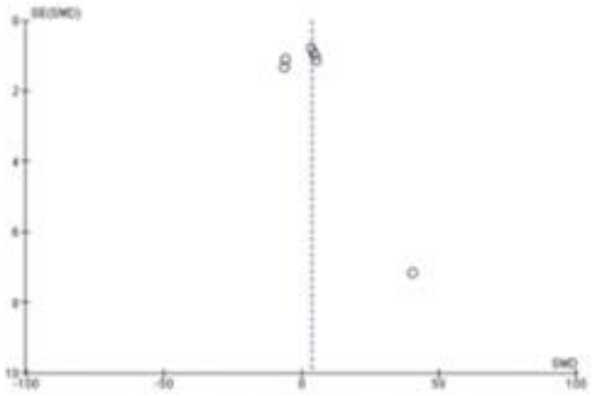
Study	Control	Sample size calculation	Material used according to manufacturer's instructions	Single operator	Amount of sealer	Time interval	Blinding of the observer	Risk of bias
Bose R et al. 2020	Y	N	Y	N	Y	Y	N	Medium
Candeiro GTM et al. 2015	Y	N	Y	N	Y	Y	N	Medium
Ehsani M et al. 2013	Y	N	Y	N	Y	Y	N	Medium
Colombo M et al. 2018	Y	N	N	N	Y	Y	N	High
Kapralos V et al. 2014	Y	N	Y	N	N	Y	N	High
Chakraborty T et al. 2020	Y	N	N	N	Y	Y	N	High
Shakya VK et al. 2016	Y	N	Y	N	Y	Y	N	Medium
Poggio C et al. 2017	Y	N	Y	N	Y	Y	N	Medium
Hasheminia M et al. 2017	Y	N	Y	N	Y	Y	N	Medium
Zordan-Bronzel CL et al. 2019	Y	N	Y	N	N	Y	N	High

Figure 2: Risk of bias assessment of the individual studies.

	Ca Si Sealer Experimental			EpoxyResin Sealer Control				Std. Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI
Bose (2) et al. 2020	6.97	0.1	9	3.81	0.77	9	18.4%	5.48 [3.25, 7.71]
Bose et al. 2020	6.45	0.09	9	3.81	0.77	9	18.6%	4.59 [2.66, 6.52]
Ehsani (2) et al. 2013	3,282	354.52	10	1,482.4	532.553	10	18.7%	3.81 [2.23, 5.39]
Ehsani et al. 2013	3,282	354.52	10	5,352	331.21	10	18.4%	-5.78 [-7.96, -3.60]
Shakya et al. 2016	11.12	0.25	10	3.64	0.013	10	7.8%	40.47 [26.45, 54.49]
Zordan-Bronzel et al. 2019	4.966	0.53	8	7.426	0.1	8	18.1%	-6.10 [-8.72, -3.47]
Total (95% CI)			**56**			**56**	**100.0%**	**3.55 [-1.54, 8.64]**

Heterogeneity: $Tau^2 = 35.39$; $Chi^2 = 134.86$, df = 5 ($P < 0.00001$); $I^2 = 96\%$
Test for overall effect: Z = 1.37 (P = 0.17)





A. Forest plot

B. Funnel plot

Figure 3: Meta-analysis with continuous variable mean difference of colony forming units. Forest plot and funnel plot for meta-analysis with standardised mean difference as variable for colony forming units.