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MARCELO AUGUSTO SERON

Influência dos cimentos obturadores biocerâmicos versus o AH Plus® na dor pós-operatória, penetração em túbulos dentinários e atividade antimicrobiana: revisões sistemáticas e meta-análises

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Orientador: Prof° Ass. Dr° Gustavo Sivieri de Araújo

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Vamos valorizar a ciência brasileira!!

"Tenho sangrado demais, tenho chorado pra cachorro
Ano passado eu morri, mas esse ano eu não morro"

BELCHIOR. "Sujeito de Sorte", Alucinação (1976)

Resumo Geral

Seron MA. **Influência dos cimentos obturadores biocerâmicos versus o AH Plus® na dor pós-operatória, penetração em túbulos dentinários e atividade antimicrobiana: revisões sistemáticas e meta-análises.** 2022. 125f [dissertação] – Faculdade de Odontologia, Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), Araçatuba, 2022.

RESUMO GERAL

O objetivo desse estudo de revisões sistemáticas e meta-análises foi responder se os cimentos biocerâmicos resultam melhores efeitos para a dor pós operatória, capacidade em penetração em túbulos dentinários e atividade antimicrobiana em comparação ao cimento AH Plus®. Foram conduzidas duas revisões sistemáticas, orientadas pelas diretrizes PRISMA e foram registradas na PROSPERO (CRD4202125928) (Capítulo 1) e na *Open Science Framework* (OSF) *Registries* (https://doi.org/10.17605/OSF.IO/BX7VQ) (Capítulo 2). Uma pergunta foi feita com base na população, intervenção, comparação e resultado (PICO), Capítulo 1: "O uso dos cimentos biocerâmicos resulta em menos dor pós operatória em comparação ao uso do cimento AH Plus® em pacientes tratados endodonticamente?"; Capítulo 2: "Os cimentos obturadores biocerâmicos apresentam superioridade na penetração em túbulos dentinários e atividade antimicrobiana ao cimento obturador AH Plus®?". Foram definidas as estratégias de buscas e realizadas buscas nas bases de dados: PubMed, Scopus, Web of Science, Embase, Cochrane Library, e OpenGrey. O Capítulo 1 utilizou a escala Cochrane para avaliar o risco de viés e a ferramenta GRADE para avaliar a qualidade das evidências. Enquanto, o Capítulo 2 utilizou a escala *The Joanna Briggs* para avaliar o risco de viés de estudos in vitro. As meta-análises foram conduzidas usando o "Meta" *package*, *version* 3.6.3, a diferença média (MD) medida de efeito foi calculada para variáveis quantitativas e odds ratio (OR) (Capítulo 1) e diferenças de médias padronizadas (SMD) (Capítulo 2), com um intervalo de confiança (IC) de 95%. Os resultados do Capítulo 1, foram incluídos 13 artigos na revisão sistemática, 11 foram incluídos na meta-análise. Para as variáveis quantitativas, o cimento biocerâmico apresentou menor ocorrência de dor pós-operatória do que o cimento AH Plus® em 24h (MD - 0,4101 [-0,80; -0,02], p = 0,0386) e 48h (MD -0,31 [-0,59; -0,03], p = 0,0295). Para as variáveis binárias, não houve diferença observada entre os cimentos avaliados: 24h (OR 1,12 [0,69; 1,80] p = 0,6476), 48h (OR 1,56 [0,76; 3,20] p = 0,2267), 72h (OR 1,38 [0,55; 3,45] p = 0,4893) e 7 dias (OR 2,10 [0,55; 8,01], p = 0,2790). Em relação à análise de risco de viés, observou-se baixo risco para a maioria dos domínios, exceto alocação que foi considerada pouco clara, enquanto a certeza da evidência variou de moderada a baixa. Os resultados do Capítulo 2, foram um total de 54 estudos foram incluídos, e 16 estudos foram incluídos na meta-análise. De modo geral, os estudos apresentaram baixo risco de viés. Não foi observado diferença estatística entre os cimentos avaliados para penetração em túbulos dentinários, independentemente dos terços: coronal SMD 0.58 [0.14; 1.31], p = 0.12; médio SMD 0.07 [0.54; 0.39], p = 0.75; e apical: SMD 0.08 [0.73; 0.56], p = 0.80. Os cimentos biocerâmicos e AH Plus® demonstraram similar ação antimicrobiana SMD [3.42; 5.32], p = 0.67 e SMD 0.67 [1.89; 0.55], p = 0.2825. Dessa forma, conclui-se que os cimentos biocerâmicos apresentam menor dor pós operatória nas primeiras 24 e 48 horas, e apresentam respostas similares para penetração em túbulos dentinários e atividade antimicrobiana quando comparado ao cimento AH Plus®.

Palavras-chaves: Anti-Infecciosos. Dor pós-operatória. Endodontia. Obturação do Canal Radicular. Revisão Sistemática. Calcarea Silicata.

Abstract

Seron MA. **Influence of bioceramic root canal sealer versus AH Plus® on postoperative pain, penetration into dentinal tubules and antimicrobial activity: systematic reviews and meta-analyses.** 125f [dissertation] – School of Dentistry, São Paulo State University (UNESP), Araçatuba, 2022.

ABSTRACT

The aim of this study of systematic reviews and meta-analyses was to answer whether bioceramic sealers have better effects on postoperative pain, ability to penetrate dentinal tubules and antimicrobial activity compared to AH Plus® sealer. Two systematic reviews, guided by PRISMA guidelines, were conducted and registered in PROSPERO (CRD4202125928) (Chapter 1) and Open Science Framework (OSF) Registries (https://doi.org/10.17605/OSF.IO/BX7VQ) (Chapter 2). A question was asked based on population, intervention, comparison and outcome (PICO), Chapter 1: "Does the use of bioceramic sealers result in less postoperative pain compared to the use of AH Plus® sealer in endodontically treated patients?"; Chapter 2: "Do bioceramic filling sealers have superior penetration into dentinal tubules and antimicrobial activity compared to AH Plus® filling sealer?". Search strategies were defined and searches performed in the following databases: PubMed, Scopus, Web of Science, Embase, Cochrane Library, and OpenGrey. Chapter 1 used the Cochrane scale to assess the risk of bias and the GRADE tool to assess the quality of evidence. Meanwhile, Chapter 2 used The Joanna Briggs scale to assess the risk of bias from in vitro studies. Meta-analyses were conducted using the "Meta" package, version 3.6.3, the mean difference (MD) effect measure was calculated for quantitative variables and odds ratio (OR) (Chapter 1) and standardized mean differences (SMD) (Chapter 2), with a 95% confidence interval (CI). The results of Chapter 1 were included 13 articles in the systematic review, 11 were included in the meta-analysis. For quantitative variables, the bioceramic sealer had a lower occurrence of postoperative pain than the AH Plus® sealer in 24 hours (MD - 0.4101 [-0.80; -0.02], p = 0.0386) and 48h (MD -0.31 [-0.59; -0.03], p = 0.0295). For the binary variables, there was no difference observed between the sealers evaluated: 24h (OR 1.12 [0.69; 1.80] p = 0.6476), 48h (OR 1.56 [0.76; 3.20] p = 0.2267), 72h (OR 1.38 [0.55; 3.45] p = 0.4893) and 7 days (OR 2.10 [0.55; 8.01], p = 0 .2790). Regarding the risk of bias analysis, a low risk was observed for most domains, except for allocation that was considered unclear, while the certainty of evidence ranged from moderate to low. The results of Chapter 2 were a total of 54 studies included, and 16 studies were included in the meta-analysis. Overall, the studies had a low risk of bias. There was no statistical difference between the sealers evaluated for penetration into dentinal tubules, regardless of the thirds: coronal SMD 0.58 [0.14; 1.31], $p = 0.12$; average SMD 0.07 [0.54; 0.39], $p = 0.75$; and apical: SMD 0.08 [0.73; 0.56], $p = 0.80$. Bioceramic sealers and AH Plus® demonstrated similar antimicrobial action to SMD [3.42; 5.32], $p = 0.67$ and SMD 0.67 [1.89; 0.55], $p = 0.2825$. Thus, it is concluded that bioceramic sealers have less postoperative pain in the first 24 and 48 hours, and have similar responses for penetration into dentinal tubules and antimicrobial activity when compared to AH Plus® sealer.

Keywords: Anti-Infectious. Calcarea Silicate. Endodontics. Postoperative pain. Root Canal Obturation. Systematic review.

Listas e Sumário

LISTA DE FIGURAS

Chapter 1

Chapter 2

D: publication bias analysis (trim-and-fill); Apical - E: Meta-analysis; F: publication bias analysis (trim-and-fill).

Figure 5. Meta-analysis antimicrobial activity. A: Meta-analysis of TotalFill BC 119
Sealer x AH Plus®. B: publication bias analysis (trim-and-fill) of TotalFill BC
Sealer x AH Plus®. C: Meta-analysis of BioRoot RCS x AH Plus®.

LISTA DE TABELAS

Chapter 1

Chapter 2

SUMÁRIO

Introdução Geral

*Normalização segundo as Normas de Vancouver. Referências Introdução Geral, p.123.

1 INTRODUÇÃO GERAL

O tratamento endodôntico tem como principal objetivo promover a descontaminação em casos de necrose pulpar ou a remoção completa do tecido pulpar em situações de polpa viva, para isso realiza-se o esvaziamento completo do sistema de canais radiculares (SCR)[1]. As estratégias utilizadas para o esvaziamento do SCR, são desde preparo biomecânico com o emprego de limas endodônticas manuais e/ou mecanizadas e a associação com soluções irrigadoras, como hipoclorito de sódio ou clorexidina[2]. Em situações de polpa necrosada há necessidade de empregar o uso de medicação intracanal de hidróxido de cálcio [3,4], promovendo redução significativa de microrganismos e seus subprodutos tóxicos[5]. Após erradicar a infecção e o completo esvaziamento do SCR, este ficará com espaços vazios, sendo necessário realizar um preenchimento com materiais obturadores endodônticos, associando o cone de guta percha com o cimento endodôntico, preenchendo toda extensão do SCR, desta forma não haverá espaços vazios e consequentemente diminui o risco de uma possível recontaminação[6].

A principal forma de avaliação da qualidade de obturação é a radiografia periapical, o que se limita a um método de 2-dimenções (2D) por meio de interpretação da radiopaciadade e radiolucidez[7], porém muitas vezes a interpretação radiográfica apresentar subjetividade entre os clínicos[8]. Com isso, Schilder[9] discutiu no passado a relação da qualidade de obturação e o índice de sucesso do tratamento endodôntico, pois sabe-se que o meio de avalição 2D sobre um objeto com 3-dimensões (3D), torna incapaz de se garantir total confiança a respeito do selamento apical e por toda extensão do SCR e de suas variações anatômicas com os materiais obturadores.

Segundo a *American Association of Endodontists*, os materiais obturadores são a associação de materiais no estado plástico, cimentos endodônticos, à materiais no estado sólido, guta percha, juntos devem ser biologicamente aceitáveis e promover o selamento do SCR[10]. Os cimentos endodônticos tem como principal função realizar a união entre a guta percha a às paredes do SCR preenchendo os espaços vazios, além de penetrar em túbulos dentinários, canais acessórios, laterais e istmos[11,12]. É sabido que há uma limitação radiográfica conforme descrito anteriormente, para determinar a qualidade de obturação do SCR, desta forma para que compense falhas na obturação que o clínico está sujeito, tem se discutido e desenvolvido novos cimentos obturadores que possam melhorar esta etapa terapêutica. Quando novos materiais obturadores surgem no mercado, há muitas dúvidas com relação a superioridade que os fabricantes tem proposto em seus materiais[11].

O pai da endodontia moderna, Dr Louis I. Grossman, descreveu quais são as características ideais que os cimentos endodônticos devem apresentar[13]. Porém, há uma vasta opção de cimentos obturadores disponíveis no mercado atualmente e nenhum deles atendem a todos requisitos citados por Grossman[13]. Desta forma é possível classificar os cimentos endodônticos por tipos, os mais populares são os cimentos endodônticos a base de óxido de zinco e eugenol, cimentos a base de hidróxido de cálcio, cimentos de ionômero de vidro, cimentos resinosos de metacrilato ou epóxi e mais recentemente os cimentos com composição de silicato de cálcio, reconhecidos como cimentos biocerâmicos[14].

O primeiro relato dos precursores dos cimentos biocerâmicos, foram do século XIX, descoberto e desenvolvido pelo dentista Dr Witte, foram chamados de cimento de Portland com aplicações odontológicas[15]. No início dos anos 90, Torabinejad e White, desenvolveram uma nova formulação a partir do cimento de Portland, que passou a ser conhecido com MTA, derivado do termo em inglês "*Mineral Trioxide Aggregate*". Esse novo material é composto por silicato tricálcico, aluminato tricálcico, óxido tricálcico, óxido de silicato e alguns outros óxidos minerais[15]. O MTA passou a ter forte aplicação na endodontia, como apexificação[16], perfuração[17] e tratamentos conservadores da polpa, como capeamento pulpar[18]. Com isso, mais recente os cimentos biocerâmicos tem a proposta aplicar na obturação do canal radicular, devido a característica do silicato tricálcico formarem hidróxido de cálcio durante o processo de presa[19].

Atualmente, o cimento endodôntico amplamente utilizado na rotina clínica é a base de resina epóxi, AH Plus® (Dentsply, DeTrey, Konstanz, Germany) por apresentar característica muito positivas desde a manipulação até as propriedades físicas, como tempo de presa, escoamento, radiopacidade, solubilidade e estabilidade dimensional[20,21]. A proposta dos cimentos biocerâmicos é apresentar características parecidas dos cimentos a base de resina epóxi, com maior biocompatibilidade, menor citotoxicidade e a capacidade de induzir resposta osteogênica com menos inflamação destacado em estudos *in vitro* e clínico[22,23]. Mas como tudo que é lançado no mercado para o uso clínico, se faz necessário realizar estudos que aprovam a sua real efetividade.

O tratamento endodôntico, pode aumentar a ansidedade do paciente e seu estresse, isso se intensifica quando há o envolvimento de dor, tornando ainda mais desafiador a rotina clínica dos endodontistas[24]. A dor pós operatória nos tratamentos endodônticos muitas vezes podem ser comuns e podem ser de origem mecânica, química ou microbiológica quando afetado o terço apical do SCR, geralmente está relacionado a consequências da instrumentação ou da obturação, independente do diagnóstico pulpar ou periapical[25,26]. A caracterização de ausência de dor pós tratamento endodôntico pode ser consideração um dos fatores para determinar o sucesso no tratamento endodôntico[27]. Mas quando presente, a dor pós obturação é inesperado e pode ser multifatorial e de curta duração[28]. Os cimentos obturadores podem levar a uma dor pós-operatória, devido sua composição química apresentar compostos que podem ser irritantes quando em contato com os tecidos perirradiculares principalmente quando há possíveis extravasamentos[26].

As propriedades físico-químicas e o poder antimicrobiana são crucias para validação de qualidade dos cimentos obturadores[13], os cimentos biocerâmicos durante o processo de presa à liberação e hidróxido de cálcio regem com os fosfatos dos fluidos dos tecidos e a consequentemente interagem por meio ligações químicas[29,30]. Outra característica, é a capacidade de penetração em túbulos dentinários, está é desejada pelos cimentos obturadores, cimentos que apresentam maior capacidade de penetração apresentam melhor retenção e aprisionar resíduos microbianos e os cimentos biocerâmicos tem se destacado nesse quesito[31,32]. A atividade antimicrobiana dos cimentos biocerâmicos, tem se destacado pelo mecanismo de liberação de hidróxido de cálcio, consequentemente alcaliniza o meio e aumenta a capacidade antimicrobiana no interior do SCR[29,33].

Assim somado a escassez de revisões sistemáticas e um consenso na literatura sobre o tema em questão, o objetivo deste trabalho foi: Capítulo 1: Avaliar se o uso dos cimentos biocerâmicos obturadores podem resultam menor dor operatória quando comparados ao cimento AH Plus®; e Capítulo 2: Avaliar se os cimentos obturadores biocerâmicos podem apresentar maior penetração em túbulos dentinários e maior atividade antimicrobiana quando comparado ao cimento obturador AH Plus®.

Chapter 1

*Standardization according to *Clinical Oral Investigations* Guidelines
https://www.springer.com/journal/784/submission-guidelines

2 CHAPTER 1 - POSTOPERATIVE PAIN AFTER ENDODONTIC TREATMENT WITH BIOCERAMICS VERSUS AH PLUS® ROOT CANAL SEALER: A SYSTEMATIC REVIEW AND META-ANALYSIS

2.1 ABSTRACT

Objectives

The aim of this systematic review and meta-analysis (SRM) was to assess the postoperative pain (PP) after endodontic treatment with bioceramic root canal sealer compared to AH Plus® sealer. The PICO question used was "Does bioceramic root canal sealer result in less postoperative pain compared with AH Plus® sealer in patients undergoing endodontic treatment?".

Materials and Methods

This systematic review followed PRISMA and was registered in PROSPERO (CRD4202125928). Searches were performed in seven databases for articles published until September 2021. The eligible criteria were randomized clinical trials (RCTs). Meta-analysis was conducted using R software with the "META" package, the mean difference (MD) measure of effect was calculated for quantitative variables and odds ratio (OR) for binary variables. The fixed effect model was applied with a 95% confidence interval (CI). The Cochrane scale was used to assess risk of bias, and the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach was used to assess the quality of evidence.

Results

Qualitative and quantitative analysis included 13 and 11 studies, respectively. For quantitative variables, the bioceramic root canal sealer presented less occurrence of postoperative pain than AH Plus® sealer in 24h (MD -0.4101 [-0.80; -0.02], p = 0.0386) and 48h (MD -0.31 [-0.59; -0.03], p = 0.0295). For binary variables, there was no difference observed between the sealers evaluated: 24h (OR 1.12 [0.69; 1.80] p = 0.6476), 48h (OR 1.56 [0.76; 3.20] p = 0.2267) ,72h (OR 1.38 [0.55; 3.45] p = 0.4893) and 7 days (OR 2.10 [0.55; 8.01], p = 0.2790). Regarding the risk of bias analysis, a low risk was observed for most domains, except allocation that was considered unclear, while the certainty of evidence ranged from moderate to low.

Conclusion

Within the limitations of study, moderate-quality evidence suggests that bioceramic root canal sealer significantly reduces the occurence of PP at 24 and 48 hours after root canal therapy. Further well conducted and standardized RCTs are needed to reassess these results.

Clinical relevance

The bioceramics sealer can be considered in clinical practice. Therefore, the endodontist must consider the sealers characteristics and your clinical experience to consider the best treatment approach.

Keywords

Bioceramic sealers, Epoxy resin-based root canal sealer, Meta-analysis, Postoperative pain, Root Canal Obturation, Systematic review

2.2 INTRODUCTION

Endodontic treatment sometimes leads to anxiety and stress for the patient, mainly when pain is involved. The postoperative pain (PP) is caused from a mechanical, chemical or microbiological injury in the apical region, appearing mainly after instrumentation and/or obturation [1]. It presents prevalence ranging from 3 to 58% [2]. Pain can be recurrent on a patient's dental routine; however, it is undesired, creates dissatisfaction and reduces the patient´s life quality [3,4]. Pain is a huge challenge for clinic routine, and it may make approaching and dealing to control and reduce symptoms difficult [5,6]. The characterization with painless post-obturation is one of the factors that lead to success in endodontic treatment [4,7]. Nevertheless, pain post-obturation, when it is presented, is unexpected and unpredictable, of multifactorial origin and of short duration [8,9], appearing mainly in the first 48 hours [10].

It is known that obturation consists of the use of thermoplasticized or cold techniques associated with gutta percha and a root canal sealer [11]. Root canal sealers are a kind of material in plastic form, and they are known on the dental market. They have different presentation shapes, with different compositions, physicochemical and biological properties [12, 13]. The root canal sealer AH Plus® (Dentsply, DeTrey, Konstanz, Germany) is considered to be the gold standard for endodontic obturation, as it gathers excellent leakage properties, radiopacity and solubility [14-17], as well as satisfactory results and closer to ideal characteristics for obturation [18]. Meanwhile, AH Plus® formula may induce inflammation process and develop pain when it is in contact to periodontal tissues [19-21].

Bioceramics sealers present on their formula calcium silicate [22]. Some studies claim they stand out for being more bioactive, biocompatible and with osteogenic induction capacity [23-28], presenting promising results in clinic [29]. Bioceramics sealers are considered to have low cytotoxicity, developing less inflammatory mediators [30] and it is suggested that they induce less PP.

Thus, due to lack of satisfactory scientific evidence and systematic reviews approaching the usage of bioceramics sealers related to occurrence of PP from endodontic treatment, this paper aimed to accomplish a systematic review and meta-analysis on randomized clinical trials (RCTs), evaluating the use of bioceramics root canal sealers results on less PP when compared to obturation with an AH Plus® sealer.

2.3 MATERIAL AND METHODS

Protocol and register

Study registration protocol was submitted to the International Prospective Register of Systematic Reviews (PROSPERO – CRD 42021259283). The systematic review and meta-analysis (SRM) was conducted under guidance of the Cochrane Handbook for Systematic Reviews of Interventions [31], and in accordance with others publications [32]. Recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) [33] were followed.

Search strategy

The electronic search was performed in the following databases: PubMed (MEDLINE), Scopus, Web of Science, Embase, Clinical Trials and Cochrane Library. Aiming to construct a search strategy, the terms Mesh (Medical Subject Heading) were used. To make the search even more sensitive, free terms that are not found by using descriptors in the databases were included (Table 1).

An expert librarian guided the entire electronic search strategy. To find unpublished or ongoing studies, clinical trials registration was investigated on the ClinicalTrials.gov website (www.clinicaltrials.gov), with no restriction on date or language of publication. In addition, gray literature (produced at government, academic, business and industrial levels, in print or electronic format, but not controlled by commercial publishers) was searched using the OpenGrey gray literature database (http://www.opengrey.eu/). A manual search was also performed to identify manuscripts that may not have been retrieved by electronic search for articles published in the following journals: Journal of Endodontics, International Endodontic Journal, Australian Endodontic Journal, Clinical Oral Investigations, Journal of Dentistry, European Endodontic Journal, and Iranian Endodontic Journal. A manual screening of reference lists from all included studies was performed, so that no relevant articles were missed. Expert authors were contacted for information about ongoing studies and unpublished data.

PICOS question and eligibility criteria

The PICOS structure (population, intervention, comparison, outcome and study design) was used to elaborate the specific question "Does the use of bioceramics root canal sealers result in less postoperative pain comparing the use of AH Plus® sealer in patients undergoing to endodontic treatment? " The PICOS strategy described below will be used:

(P) — Population: patients who have received conventional endodontic treatment;
(I) —Intervention: bioceramics root canal sealers;
(C) - Comparison: AH Plus® root canal sealers;
(O) – Outcome: postoperative pain; and
(S) – Study design: randomized clinical trials.

For this SRM, studies that met the following inclusion criteria were selected: (a) RCTs that evaluated PP in conventional root canal treatment using bioceramics root canal sealers (intervention) versus AH Plus® root canal sealers (comparison); (b) studies with a follow-up period between immediately after treatment and seven days later, at most; (c) patients without complicating changes or systemic diseases; (d) studies with patients without severe pain and/or acute apical abscess and and (e) studies with 10 patients (n=10) per group, at least. Prospective or retrospective studies, case series, case reports, non-human studies, literature review articles or expert opinions were excluded.

Studies' selection

In the first step, all references registered in all databases were imported into online reference management websites (EndNote Web; Thomson Reuters Inc., Philadelphia, PA, USA). After eliminating duplicates, two reviewers (M.A.S. and G.P.N.) independently assessed all titles and abstracts. In cases of titles and abstracts with insufficient information, the full texts were analyzed. Any differences were solved through discussion and a third author (G.S.A.) was consulted when necessary. Agreement between the two reviewers regarding title and abstract selection was assessed using Cohen's kappa coefficient (ϰ). Subsequently, the full texts of the other articles were analyzed and those that met the inclusion criteria were included.

Data extraction process

Details associated with the study were acquired through customized extraction forms, on which the following parameters were recorded: study details (authors, year of publication and location), intervention groups (n), sex and age, type of root morphology, clinical condition / diagnosis, preoperative condition, pain analysis method, follow-up periods, use of medications, outcomes. When necessary, the authors were contacted by email to be requested additional information.

Risk of bias of included studies

Two authors (M.A.S. and G.P.N.) independently assessed the risk of bias of the selected studies using the Cochrane Collaboration's tool to assess the risk of bias of RCTs [34]. The risk of bias tool contains six domains: sequence generation, allocation concealment, masking of outcome assessors, incomplete outcome data, selective outcome reporting, and other possible sources of bias. Each domain was assessed as having low, unclear, or high risk of bias according to the Cochrane Handbook for Systematic Reviews of Interventions 5.1.0 (http://handbook.cochrane.org). Conversely, when a study was judged to be unclear in its key domains, its authors were contacted in order to provide more information to allow a definitive judgment of "yes" or "no". If the final judgment remained dubious for one or more key domains, the studies were considered to be at risk of bias [34].

If blinding was applied to the patients, the domains "blinding of participants and personnel" and "blinding of the outcome" were considered to be of low risk, since the endodontist cannot be blinded to the procedure (type of sealer used) and the patients themselves recorded their pain experience on a visual analogue scale (VAS).

Summary measurements

Quantitative analyses were performed using the R software with “Meta” package, version 3.6.3 to assess PP in bioceramics sealers versus AH Plus® sealer in root canal treatment. For quantitatively measured results, measure of effect was used between the means difference (MD), and for the calculation of meta-analysis, the inverse variance method was used. As for binary results, the measure of effect used was odds ratio. Additionally, as binary results presented sparse data (sparce data), the "Peto" method was used to carry out the meta-analysis. Meta-analyses were performed according to the reported evaluation period: quantitative data (24 and 48 hours) and binary data (24, 48, 72 hours and 7 days). In both analyses, the fixed-effect model was applied with a 95% confidence interval (CI). Heterogeneity was tested using the I^2 index, and an I^2 index $\geq 50\%$ was considered substantial or high. To check publication bias, the trim-and-fill method ($n > 3$) was used.

Quality evaluation of evidences by Grading of recommendations, assessment, development and evaluation (GRADE)

The quality of evidence was graded for each outcome in the studies (body of evidence) using the GRADE tool (http://www.gradeworkinggroup.org/) to determine the overall strength of the evidence if there was a meta-analysis. The GRADE approach was used to contextualize or justify intervention recommendations with four levels of quality of evidence, ranging from high to very low.

The GRADE approach starts with a study design (RCTs or observational studies) and then addresses five reasons (risk of bias, imprecision, inconsistency, indirect evidence and publication bias) to possibly decrease the quality of evidence (1 or 2 levels) and three to possibly assess quality (large effect; management of confounding factors; dose-response gradient) [35,36]. Each of these topics was rated 'unlimited'; 'Serious Limitations' and 'Very Serious Limitations' to allow categorization of the quality of evidence for each outcome into high, moderate, low and very low. "High quality" suggests that it has big chances of true effect that will be close to the effect estimate. On the other hand, "very low quality" suggests that chances are low in the effect estimate and the reported estimate may be substantially different from what was measured. The evaluations were carried out by two researchers independently, (G.P.N. and M.A.S.) and later compared.

2.4 RESULTS

Literature search

The search in the databases collected 579 studies: 139 from PubMed/MEDLINE, 159 from Scopus, 76 from Web of Science, 43 from Embase, 155 from the Cochrane Library and 7 by manual searches (Figure 1). After removing the duplicates, 309 studies remained for evaluation of titles and abstracts, with 293 studies being excluded for not meeting the eligibility criteria. It resulted in 16 studies for full reading, with 3 studies excluded for not having a control group. Thus, 13 studies of RCTs were included in the inclusion criteria. Kappa scores for articles included in all databases showed an acceptable level of agreement among examiners (k = 0.93).

Description and features of included studies

The data extracted from the 13 studies included are detailed in Table 2. A total of 1,006 patients with a mean age of approximately 41.34 years were evaluated in the studies, with 478 individuals undergoing endodontic treatment with the AH plus® sealer (control group) and 528 individuals with the bioceramics sealers (intervention). All studies evaluated only patients without complicating changes or systemic diseases. Regarding the origin of the studies, three studies were carried out in Brazil [37-39], two in India [40,41] and two in Turkey [42,43], and one in each country, USA [44], South Korea [45], Singapore [1], Spain [46], Portugal [47] and Lithuania [48].

Regarding the number of roots, five studies analyzed single-rooted teeth [38-41,48], three studies were performed with multirooted teeth [37,42,44] and five studies used both tooth morphologies [1,43,45-47]. Related to pulp and periapical diagnosis, three studies included patients with apical periodontitis [40,46,48], four studies included patients with irreversible pulpitis [37,41,42,44], two with pulp necrosis [38,39] and four with samples in both conditions: irreversible pulpitis and pulp necrosis [1,43,45,47].

The VAS was the most used pain assessment method in the selected studies [38-48]. The follow-up period for PP was quite extensive, ranging from immediate obturation of the root canals to 7 days after completion of the endodontic treatment. In general, the greatest cases of pain were found when teeth were symptomatic prior to endodontic therapy. Typically, the greatest pain time occurred in the first hours up to 48 hours after endodontic treatment, and the smallest time was reported after 72 hours of the follow-up. Analgesic consumption was a condition assessed in 10 out of 13 studies [1,37-40,42-44,47,48], which was prescribed to patients only in cases of severe pain; five studies reported that ibuprofen was the prescribed medication [38,39,42,43,47], two studies prescribed the acetaminophen [37,44] and three studies did not specify which medication was used [1,40,48].

Risk of bias in included studies

The assessment of risk of bias in the selected studies is shown in Table 3. Considering risk of bias, according to the Cochrane scale, 8 out 13 studies were classified as low [1,38-40,42,43,45,48], four as unclear [37,44,46,47] and one as high [41].

RCTs demonstrated “low risk of bias” for the random sequence domain (selection bias), except for three studies [41,44,47] that had an “uncertain risk of bias”. Five studies did not report sufficient information for allocation concealment, being considered as “uncertain risk of bias” [37,41,44,46,47]. One study showed "high risk of bias" for data domains of selective outcome reporting and other sources of bias [47], because it did not report pain intensity data, and because it presented flaws in its study design and lack of clear information about applied methodology, respectively.

Synthesis meta-analysis and level of evidence

In the meta-analysis performed with quantitative variables, endodontic treatment performed with bioceramics sealers showed lower occurrence of PP in 24 hours: MD -0.4101 [-0.80; -0.02], $p = 0.0386$ (Fig. 2A) and 48h: MD -0.31 [-0.59; -0.03], $p = 0.0295$ (Fig. 2C). In addition, after a 24-hour period, the trim-and-fill test identified three articles with possible publication bias [40,45,48], but no significant results were found ($p = 0.3790$) (Fig. 2B). On both evaluation periods, the evidence quality was classified as moderate (Table 4).

For binary variables, no statistically significant results were found among the sealers analyzed in all analyses periods 24 hours, 48 hours, 72 hours, and 7 days: OR 1.12 [0.69; 1.80], $p = 0.6476$ (Fig. 3A); OR 1.56 [0.76; 3.20], $p = 0.2267$ (Fig. 3C); OR 1.38 [0.55; 3.45], $p = 0.4893$ (Fig. 3E) and OR 2.10 [0.55; 8.01], $p = 0.2790$ (Fig. 3F), respectively. A low level of heterogeneity was observed, and publication and meta-analysis bias was only observed for the 48-hour period in 2 studies [38,47], though without significance (p = 0.6990) (Fig. 3B). The quality of evidence for the 24-hour period was determined as moderate and, for the other periods (48 hours, 72 hours and 7 days), as low (Table 4).

2.5 DISCUSSION

This SRM aimed to evaluate the occurrence of PP due to the type of root canal sealer used: bioceramics sealers (intervention) versus the AH Plus® sealer (control). According to the meta-analyses performed in this study, for quantitative data, in the periods of 24 hours (I^2=36%; p= 0.0386) and 48 hours (I^2=48%; p= 0.0295), the group of bioceramics sealers had a lower incidence of PP compared to the control group (AH® Plus sealer). It is important to notice that the first 48 hours after surgery is the period in which pain is most present [2,49,50], thus, the effect observed in these assessment periods is justifiable by the greater report of pain in the patient's own subjective analysis. Furthermore, it is suggested that the bioceramics sealers have a shorter setting time, better cell viability and cell migration capacity compared to AH Plus® [19,51]. In addition to this, it is also suggested that bioceramics have less activation of nociceptors responsible for calcitonin gene release that leads to neurogenic inflammation and pain [20]. Some bioceramics sealers (MTA Fillapex and EndoSequence BC Sealer) showed low expression of inflammatory markers, such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α), showing less inflammatory activity [30]. AH Plus® is mainly composed of epoxy resin and amine paste, and it was observed in previous studies that these components can produce inflammatory effects and generate pain [19,20], mainly due to release of monomers such as ether bisphenol-A-diglycidyl [52,53].

On the other hand, the meta-analyses performed with binary data did not show any statistical difference between the root canal sealers evaluated in any postoperative period. Thus, in both variables (binary and quantitative data) there was also no difference between sealers for pain levels for periods longer than 48 hours. This data reinforce findings that PP gradually decreases over time [32,54,55], and possibly due to this decline resulting from patient reports (subjective) there was lower accuracy power to verify differences in effect estimates. Furthermore, nine studies reported rescue medication prescription with analgesics [1,37-40,42-44,47,48]; only two studies [1,39] reported the amount of patients who used the drug; and there was no difference between types of root canal sealer evaluated.

Pain is subjective, as it is measured through reports and pain thresholds peculiar to each individual. Moreover, studies that have PP as an outcome are usually difficult to analyze and to establish an interpretation [2,56]. As an analysis variable, the most indicated scale for evaluating PP studies is VAS; it is considered the safest and most reliable in determining the results [57], and they are consolidated in the endodontic literature [2,58]. For these reasons the meta-analyses were performed only with studies that used this method of assessment, as well as to reduce factors linked to heterogeneity. In contrast, the Likert-type scale adopts a method considered to be push-factor choice, which may be a study variable due to the reliability of results [57].

In addition, pain presents itself with great variability during all stages of the endodontic treatment. The presence of preoperative pain is related to the patient's demographic characteristics, such as age, sex and type of dental element analyzed; intraoperative pain, such as obturations techniques and type of endodontic sealer; PP, such as anesthesia and treatment time [59]. The occurrence of mild PP is common, even when endodontic treatment is within acceptable standards [60]. Furthermore, pain is related to chemical, mechanical and microbial damage, and the inflammatory response is directly proportional to the intensity of damage to periapical tissues [60]. The initial endodontic diagnosis is a variable in studies for analysis of PP, especially in cases of irreversible pulpitis and acute apical periodontitis, especially in the first postoperative hours [58]. These factors increase the patient's probability of pain after treatment with different intensities [8,58].

Some studies included in this SRM evaluated the unintentional apical extrusion of AH Plus® and bioceramics sealers; Sealer Plus BC [39] and EndoSequence BC Sealer, and Bio-C Sealer [37] obtained results wasn't affected to sealer type on evaluation of PP. This result [37,39] is possibly due to the fact that the small amounts of root canal sealer that were extruded were not enough to promote a significant inflammatory response in periapical tissues. These findings reinforce the hypothesis that only the factor of choice of root canal sealer does not influence PP significantly, even in clinical situations with small unintentional apical extrusion [37,39]. On the other hand, it is necessary to be careful with extravasation of root canal sealers, since the contact of obturation material with the periapical tissues can lead to an inflammatory response [59], especially in lower molars, as, in most cases, the root periapex is close to the mandibular canal and may cause nervous tissue injury [59]. Thus, this is a variable that requires further clinical studies, especially in the case of endodontic therapy with AH Plus® sealer, as this sealer produces toxic monomers that can increase oxidative stress in human cells, which may be associated with the release of reactive oxygen species responsible for tissue inflammation [61], and their extrusion can delay periapical repair [62]. Furthermore, the American Association of Endodontists [63,64] indicates that the obturation should preferably remain inside the root canal, and these are factors that can influence successful treatment.

Five studies in this systematic review used thermoplastic obturation equipment [1,37,43,44,48]. Thermoplasticized obturation techniques did not influence PP, even though it was initially assumed that the thermocompressor equipment could damage the periodontium due to instrument heating reaching 200° degrees [65,66]. On the other hand, there is the hypothesis that the blood flow of the periodontium acts as a protector for heating of these devices [67], which corroborates the fact that obturatiomn technique may not exert much influence on incidence of post-obturation pain [44]. A recent study has reported [68] that the thermoplastification technique, when used in bioceramics sealers (EndoSequence BC, Bio-C, and BioRoot sealers), can change and compromise the setting time and dimensional stability of the sealer, as well as reduce its antimicrobial effect [69] and allow more extravasation of obturation materials [70]. In short, it is suggested that, in the long term, the quality of root canal obturation may be compromised [68]. Thus, it is necessary to pay attention to the physicochemical properties, as well as to conduct more clinical trials evaluating these parameters, as heated obturation techniques can influence the properties of bioceramics sealers [71] and has not been evaluated in this meta-analysis due to the lack of sufficient information in the eligible study groups.

Regarding the quality assessment of this systematic review, five of the articles included [37,41,44,46,47] showed an uncertain risk of bias for the domains of sequence generation and/or allocation concealment. It is assumed that influence of selection process, as well as the allocation process, can have a significant impact on the results, as both variables can represent confounding factors. In addition, potential biases associated with inadequate allocation concealment can lead to an overestimation of the treatment effect and influence the results of the meta-analysis [72,73]. Hence, the GRADE approach and quality assessment were carried out as an alternative strategy to avoid overestimating the results with a conservative approach in the description. In the present review, according to the GRADE approach, the meta-analysis for quantitative data and for the 24-hour period for binary data showed moderate evidence of certainty, with the inconsistency domain downgraded due to substantial heterogeneity and variability of results from the selected studies. As for the other postoperative periods (binary variables), the evidence was classified as low, suggesting that the true effect may not be confirmed due to inconsistent results and imprecision [35]. Thus, the heterogeneity between the findings of the included studies and the inclusion of few patients and few events and a wide confidence interval (CI) around the effect estimate were responsible for the low estimated evidence effect.

The methodological variability between studies and difficulties in presenting their results in a standardized way are limitations of this systematic review. These aspects are associated with the number of studies included in the meta-analysis, making it impossible to analyze the influence of the variables in isolation. In addition, the sample size of most studies was very small, which may have led to an overestimation of the intervention's effects. The importance of standardizing variables should be taken into account in more robust future clinical trials, with the objective of reducing potential confounding factors and enabling an individualized analysis of these factors in terms of PP. These also include pulpal and/or periapical condition, preoperative pain status, evaluation of the number of rescue analgesics consumed postoperatively, obturation techniques, obturator sealer extrusion. Our results demonstrated that bioceramics sealers can reduce PP in the first 48 hours. Under the fixed-effect model, it was assumed that studies used in the meta-analysis share a common effect size and factors that could influence the effect size are the same across studies, varying from one study to another because of random error inherent to each study.

Finally, although the results suggested a lower occurrence of PP for bioceramics sealers, certainty of evidence shown in this research indicates that well-defined parameters and more precise control of variables are still necessary for their choice of first choice in clinical practice. This review encourages the conduct of new laboratory studies and future well-designed and robust clinical trials for definitive recommendations.

2.6 CONCLUSION

Even with study limitations, bioceramics sealers reduced postoperative endodontic pain only after 24 and 48 hours compared to the AH Plus® sealer, with moderate certainty of evidence. However, more robust and standardized clinical trials are needed to confirm the results with less heterogeneity and higher quality of evidence.

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Table 1. Search strategy

Search Strategy
#1 (Root Canal Obturation) OR (Canal Obturation, Root) OR (Canal Obturations, Root) OR (Obturation, Root Canal) OR (Obturations, Root Canal) OR (Root Canal Obturations) OR (Endodontic Obturation) OR (Endodontic Obturations) OR (Obturation, Endodontic) OR (Obturations, Endodontic) OR (Sealer-Based Obturation)
#2 (Pain, Postoperative) OR (Post operative Pain) OR (Postsurgical Pain) OR (Post-operative Pain) OR (Post-operative Pains) OR (Postoperative Pain) OR (Post-surgical Pain) OR (Pain, Post-surgical) OR (Post surgical Pain) OR (Pain, Post-operative) OR (Pain, Post operative) OR (Pain, Postsurgical) OR (Postoperative Pain, Chronic) OR (Pain, Chronic Postoperative) OR (Chronic Postoperative Pain) OR (Chronic Post-surgical Pain) OR (Chronic Post surgical Pain) OR (Pain, Chronic Post-surgical) OR (Post-surgical Pain, Chronic) OR (Chronic Postsurgical Pain) OR (Chronic Postsurgical Pains) OR (Pain, Chronic Postsurgical) OR (Postsurgical Pain, Chronic) OR (Persistent Postsurgical Pain) OR (Pain, Persistent Postsurgical) OR (Postsurgical Pain, Persistent) OR (Post-operative Pain, Chronic) OR (Pain, Chronic Post-operative) OR (Post operative Pain, Chronic) OR (Chronic Post-operative Pain) OR (Chronic Post operative Pain) OR (Postoperative Pain, Acute) OR (Pain, Acute Postoperative) OR (Acute Postoperative Pain) OR (Acute Post-operative Pain) OR (Acute Post operative Pain) OR (Post-operative Pain, Acute) OR (Pain, Acute Post-operative) OR (Post operative Pain, Acute) OR (Postobturation Pain) OR (Dor Pós-Operatória) OR (Pain, Postoperative)
#1 AND #2

Table 2. General data on the selected studies

Author/ year (City, Country)	G1 - AH Plus Group G2 - Bioceramic Group (N patients)	Sex (Male/Female) and Age (y)	Tooth Type	Clinical Condition/ Diagnosis Pre-op Status	Pain Assessments	Pain Evaluation Periods	Medication Use (n patients)	Outcome: Postoperative Pain [Mean ± SD] or Percentage
Drumond et al., 2021 (Campinas, Brazil) [37]	G1 (AH Plus): 13 G2.1 (Endo Sequence BC Sealer): 13 G2.2 (Bio-C Sealer): 13 Vertical compaction - Schilder's technique (Odous touch®)	G1: 7/6 Age: 34.4 ± 7.6 G2.1: 6/7 Age: 32.2 ± 10.3 G2.2: 6/7 Age: 33.3 ± 10.2	Multi	Asymptomatic Irreversible Pulpitis	VDS	6h, 12h, 24h, 48h and 7 days	If Necessary, 500-mg Acetaminophen	G1: 6h: 0.77 ± 1.2 12h: 0.62 ± 1.1 24h: 0.15 ± 0.4 48h: 0.23 ± 0.8 7 days: 0.0 ± 0.0 G2.1: 6h: 1.23 ± 1.6 12h: 0.77 ± 1.2 24h: 0.54 ± 1.3 48h: 0.69 ± 1.7 7 days: 0.15 ± 0.4 G2.2: 6h: 1.08 ± 1.8 12h: 1.00 ± 1.6 24h: 0.08 ± 0.3 48h: 0.00 ± 0.0 7 days: 0.00 ± 0.0
	G1 (AH Plus): 19	G1: 15/7 Age: 41.68					Only as Needed	G1 24h: 11.57 ± 11.18 48h: 1.57 ± 3.74 72h: 0.00 ± 0.00

Khandelwal et al., 2021 (Chennai, India) [40]	G2 (BioRoot RCS): 19	G2: 11/8 Age: 43.63	Single	Necrotic Pulp with Chronic Apical Periodontitis	VAS	24h, 48h, 72h and 7 days		Day 7: 0.00 ± 0.00 G2 24h: 4.73 ± 7.72 48h: 1.57 ± 3.74 72h: 0.00 ± 0.00 Day 7: 0.00 ± 0.00
Yu et al., 2021 (Philadelphia, USA) [44]	G1 (AH Plus): 41 Warm Vertical Compaction Technique G2 (Endo Sequence BC Sealer): 51 Single Cone Gutta Percha	G1: 11/30 Age: 46.2 ± 14.4 G2: 17/34 Age: 46.7 ± 16.6	Single and Multi	Symptomatic or Symptomatic Irreversible Pulpitis	VAS	4, 24 and 48 h	1000mg Acetaminophen (Only as Needed)	G1: 4h: 2.41 ± 2.21 24h: 1.95 ± 2.22 48h: 1.15 ± 1.80 G2: 4h: 2.27 ± 2.35 24h:1.80± 2.47 48h: 1.00 ± 1.66
Shim et al., 2021 (Seoul, Republic of Korea) [45]	G1: 32 AH Plus (Continuous-wave Technique) Anterior Teeth or Premolars: 15; molars:17 G2: 35	Age: 49.04±16.62 years	Single	G1 /G2 Pulp Diagnosis: Vital: 18 / 17 Non Vital: 14 / 18 Pre-periapical Lesion: Present: 20 /18 Absent: 12 / 17	VAS	Over the 7 day	NR	G1 / G2 Anterior teeth 1 day: 0.0 ± 8.48 / 6.0 ± 4.59 2 day: 1.0 ± 7.07 / 4.0 ± 8.13 72h up to 7 days G1 and G2 = 00.0 ± 0.00 Molars teeth 1 day: 5.0 ± 5.65 / 0.5 ± 5.30 2 day: 3.5 ± 4.94 / 0.0 ± 3.88

	Endoseal MTA (Single-cone Technique): Anterior Teeth or Premolars: 17; Molars:18			Pre-operative pain: Present: 14 / 13 Absent: 18 / 19				3 day: 3.0 ± 5.30 / 0.0 ± 3.88 4 day: 2.5 ± 4.59 / 0.0 ± 3.32 5 day: 3.0 ± 4.45 / 0.0 ± 3.53 6 day: 3.0 ± 4.24 / 0.0 ± 3.53 7 day: 0.0 ± 0.0 / 0.0 ± 0.0
Tan et al., 2021 (Singapore) [1]	G1 (AH Plus): 83 G2 (Total Fill BC): 80 Heated Plugger (System B)	Patients Aged 21 years and Older	Single and Multi	Vital, Nonvital, and Previously Root-Filled Teeth	Incidence of Pain Likert Scale	1, 3 and 7 days	G1: 2 G2: 2	Count (%) Pain G1: Day 1: 16 events of 83 (19.3%) Day 3: 8 events of 83 (9.6%) Day 7: 3 events of 83 (3.6%) G2: Day 1: 18 events of 80 (22.5%) Day 3: 8 events of 80 (10.0%) Day 7: 6 events of 80 (7.5%)
				G1 /G2				G1: Immediate post op: 0 ± 1.95 8h: 0 ± 1.87 24h: 0.0 ± 0.0 48h: 0.0 ± 0.0 G2: Immediate post op: 0 ± 2.65 8h: 0 ± 2.45 24h: 0.0 ± 0.0

								48h: 0.0 ± 0.0
Tousif Iqbal Nathani, 2020 (Catalunya, Spain) [46]	G1 (AH Plus): 34 Vertical Condensation of Warm Gutta-Percha G2 (BioRoot RCS): 41 Single Master Cone Technique	Patients aged 18 years and older	Single and Multi	Symptomatic Apical Periodontitis 7 / 16 Asymptomatic Apical Periodontitis 13 / 10	VAS	8, 24 and 48 h	NR	Count (%) Pain G1 Immediate post op: 14 events of 34 (41%) 8h: 12 events of 34 (35%) 24h: 6 events of 34 (17%) 48h: 2 events of 34 (5%) G2 Immediate post op: 19 events of 41 (46%) 8h: 20 events of 41 (48%) 24h: 7 events of 41 (17%) 48h: 6 events of 41 (14%)
Ferreira et al., 2020 (Pelotas, Brazil) [38]	G1 (AH Plus): 20 G2 (MTA Fillapex): 20 The Single Cone and Vertical Compaction Technique	G1: 4/16 G2: 7/13 Patients Older than 18 years	Single	Pulp Necrosis	Incidence of Pain	24 h, 48 h and 7 days	600 mg Ibuprofen (only as needed)	G1: 24h: 3 events of 20 (15%) 48h: 1 event of 20 (5%) 7 days: 0 events of 20 (0%) G2: 24h: 3 events of 20 (15%) 48h: 3 events of 20 (15%) 7 days: 0 events of 20 (0%)

Pandey et al., 2020 (Davangere, India) [41]	G1 (AH Plus): 17 G2 (BioRoot RCS): 17	18 to 65 years	Single	Irreversible Pulpitis Without Apical Periodontitis	VAS	24h and 48h	NR	G1 Preop pain: 6.12 ± 1.58 24h: 1.82 ± 2.13 48h: 0.65 ± 1.46 G2 Preop pain: 6.06 ± 1.56 24h: 1.35 ± 1.94 48h: 0.71 ± 1.69
Aslan et al., 2020 (Kayseri, Turkey and Aydın, Turkey) [42]	G1: (AH Plus): 30 G2.1: (Endoseal MTA): 30 G2.2: (EndoSequence BC Sealer): 30 The Single Gutta-Percha Cone	G1: 10/16 37.15 ± 11.93 G2.1: 6/22 39.57 ± 13.09 G2.2: 12/18 32.46 ± 13.20	Multi	Asymptomatic Irreversible Pulpitis	VAS	6h, 12h, 24h, 48h, 3 days, 4 day, 5 day, 6 day and 7 day	600 mg Ibuprofen (Only as Needed)	G1: 6h: 30.0 12h: 20.0 24h:10.0 G2.1: 6h: 20.0 12h: 10.0 24h: 2.0 G2.2: 6h: 30.0 12h: 20.0 24h:10.0 48h up to 7 days G1; G2.1 and G2.2 = 00.0 ± 0.00
	G1 (AH Plus): 32 G2 (Sealer Plus BC): 32	G1: 14/18 37.09 ± 13.10	Single	Pulp Necrosis	VAS	24 h, 48 h, 72 h and 7 days	G1: 1 G2: 2	G1: 24h: 1.46 ± 1.96 48h: 0.44 ± 0.86 72h: 0 ± 0.0 7 days: 0 ± 0.0

Fonseca et al., 2019 (Campinas, Brazil) [39]	The single gutta-percha Cone	G2: 12/20 38.5 ± 14.18					600 mg Ibuprofen (Only as Needed)	G2: 24h: 1.21± 2.09 48h: 0.09 ± 0.38 72h: 0 ± 0.0 7 days: 0 ± 0.0
Atav Ates et al., 2018 (Adana, Turkey) [43]	G1 (AH Plus): 80 Vital: 40 Devital: 40 G2 (iRoot SP): 80 Vital: 40 Devital: 40 Herofill™ Soft-Core Obturators (Micro-Mega)	G1: Vital: 16/23 Age: 30.69 ± 10.39 Devital: 15/24 Age: 36.33 ± 11.08 G2: Vital: 17/22 Age: 35.00 ± 12.55 Devital: 19/20	Single and Multi	Vital and Devital	VAS	6 h, 12 h, 24 h, and 72 h	200 mg Ibuprofen (Only as Needed)	G1 Vital: 0h: 4.05 ± 3.74 6h: 2.7 ± 2.8 12h: 2.0 ± 2.8 24h: 0.9 ± 1.6 72h: 0.2 ± 0.7 G2 Vital: 0h: 4.03 ± 3.69 6h: 2.5 ± 2.5 12h: 1.5 ± 2.5 24h: 0.7 ± 1.5 72h: 0.3 ± 0.9 G1 Devital: 0h: 3.31 ± 3.64 6h: 2.7 ± 3.3 12h: 1.6 ± 2.1 24h: 1.2 ± 2.1 72h: 0.2 ± 0.5 G2 Devital: 0h: 3.10 ± 4.14 6h: 1.7 ± 2.2 12h: 1.2 ± 1.9 24h: 0.6 ± 1.3

		Age: 40.69 ± 11.87						72h: 0.3 ± 1.4
Paz et al., 2018 (Lisbon, Portugal) [47]	G1.1 (AH Plus + Continuous Wave of Condensation): 10 G1.2 (AH Plus + Cold Lateral Condensation): 10 G2 (BioRoot RCS + Single Cone Technique): 10	NR	Single and Multi	Irreversible Pulpitis, Pulp Necrosis and Endodontic Retreatment	VAS	24h, 48h, 72h, 96h, 120h, 144h and 168h	600 mg Ibuprofen (Only as Needed)	G1 24h: 5 events of 20 (25%) 48h: 3 events of 20 (15%) 72h: 2 events of 20 (10%) 96h: 1 event of 20 (5%) 120h: 1 event of 20 (5%) 144h: 0 events of 20 (0%) 168h: 0 events of 20 (0%) G2 24h: 5 events of 10 (50%) 48h: 4 events of 10 (40%) 72h: 3 events of 10 (30%) 96h: 1 event of 10 (10%) 120h: 1 event of 10 (10%) 144h: 0 events of 10 (0%) 168h: 0 events of 10 (0%)
								Mean ± SD Patients with Postoperative Pain G1 24h: 21.67 ± 20.38 48h:15.56 ± 10.14

Graunaite et al., 2018 (Kaunas, Lithuania) [48]	G1 (AH Plus): 57 G2 (Total Fill BC): 57 Warm Vertical Condensation Technique (Calamus Dual System)	Age: 49.5 ± 12.82 years	Single	Asymptomatic Apical Periodontitis	VAS	24 h, 48 h, 72 h, and 7 days	Unspecified Analgesic (Only as Needed)	G2 24h: 18.0 ± 19.32 48h: 11.43 ± 3.78 General Pain in Total Patients G1 24h: 4.56 ± 12.69 48h: 2.46 ± 6.89 G2 24h: 3.16 ± 10.38 48h: 1.40 ± 3.98 Pain G1 24h: 12 events of 57 48h: 9 events of 57 G2 24h:10 events of 57 48h: 7 events of 57

Table 3. Risk of Bias of Randomized Controlled Trials - Cochrane Scale

	Sequence Generation	Allocation Concealment	Blinding of Participants, Personnel	Blinding of Outcome Assessors	Incomplete Outcome Data	Selective Outcome Reporting	Other Sources of Bias
Drumond et al. 2021 [37]	Low	Unclear	Low	Low	Low	Low	Low
Khandelwal et al. 2021 [40]	Low	Low	Low	Low	Low	Low	Low
Shim et al. 2021 [45]	Low	Low	Low	Low	Low	Low	Low
Tan et al. 2021 [1]	Low	Low	Low	Low	Low	Low	Low
Yu et al. 2021 [44]	Unclear	Unclear	Low	Low	Low	Low	Low
Aslan et al. 2020 [42]	Low	Low	Low	Low	Low	Low	Low
Ferreira et al. 2020 [38]	Low	Low	Low	Low	Low	Low	Low
Pandey et al. 2020 [41]	Uncler	Unclear	Low	Low	Low	High	High
Tousif Abqal Nathani. 2020 [46]	Low	Unclear	Low	Low	Low	Low	Low
Fonseca et al. 2019 [39]	Low	Low	Low	Low	Low	Low	Low
Atav Ates et al. 2018 [43]	Low	Low	Low	Low	Low	Low	Low
Graunaite et al. 2018 [48]	Low	Low	Low	Low	Low	Low	Low
Paz et al. 2018 [47]	Unclear	Unclear	Low	Low	Low	Low	Unclear

Table 4. Evidence profile Postoperative pain in endodontic treatment in bioceramic cement vs. AH Plus® cement

Certainty Assessment							№ of Patients		Effect	Certainty
№ of Studies	**Study Design**	**Risk of Bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other Considerations**	**[Bioceramic]**	**[AH Plus®]**	**Absolute (95% CI)**	
Quantitative variables										
Pain (24h)										
8	randomised trials	not serious	serious	not serious	not serious	none	274	263	**MD 0.41 SD higher (0.80 lower to 0.02 lower)**	⊕⊕⊕◯ MODERATE
Pain (48h)										
6	randomised trials	not serious	serious	not serious	not serious	none	194	183	**MD 0.31 SD higher (0.59 lower to 0.03 lower)**	⊕⊕⊕◯ MODERATE
Binary variables										
Pain (24h)										
5	randomised trials	not serious	serious	not serious	not serious	none	208	214	**MD 1.12 SD lower (0.69 lower to 1.80 lower))**	⊕⊕⊕◯ MODERATE

Certainty Assessment							№ of Patients		Effect	Certainty
№ of Studies	**Study Design**	**Risk of Bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other Considerations**	**[Bioceramic]**	**[AH Plus®]**	**Absolute (95% CI)**	
Pain (48h)										
4	randomised trials	not serious	serious	not serious	serious	none	128	131	**MD 1.56 SD higher (0.76 lower to 3.20 lower)**	⊕⊕◯◯ LOW
Pain (72h)										
2	randomised trials	not serious	serious	not serious	serious	none	90	103	**MD 1.38 SD lower (0.55 lower to 3.45 lower)**	⊕⊕◯◯ LOW
Pain (7days)										
3	randomised trials	not serious	serious	not serious	serious	none	110	123	**MD 2.10 SD higher (0.55 lower to 8.01 lower)**	⊕⊕◯◯ LOW

Figure 1. PRISMA Flowchart – Flow diagram showing the entre search process

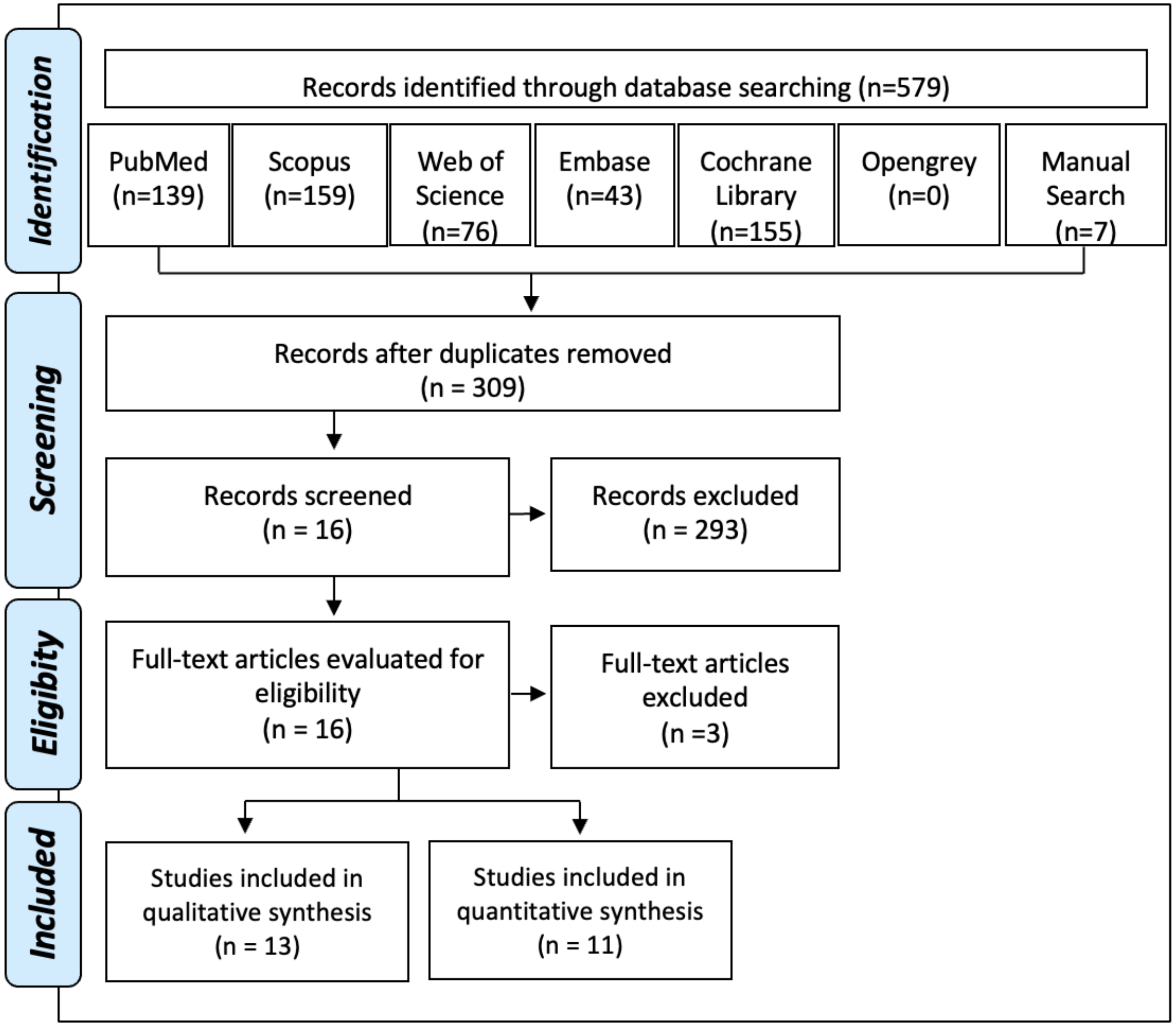


Figure 2. Meta-analysis quantitative variables for the periods of 24h and 48h. A: Meta-analysis of 24h. B: Analysis of publication bias and meta-analysis of 24h (trim-and-fill). C: Meta-analysis of 48h. D: Analysis of publication bias and meta-analysis of 48h (trim-and-fill)

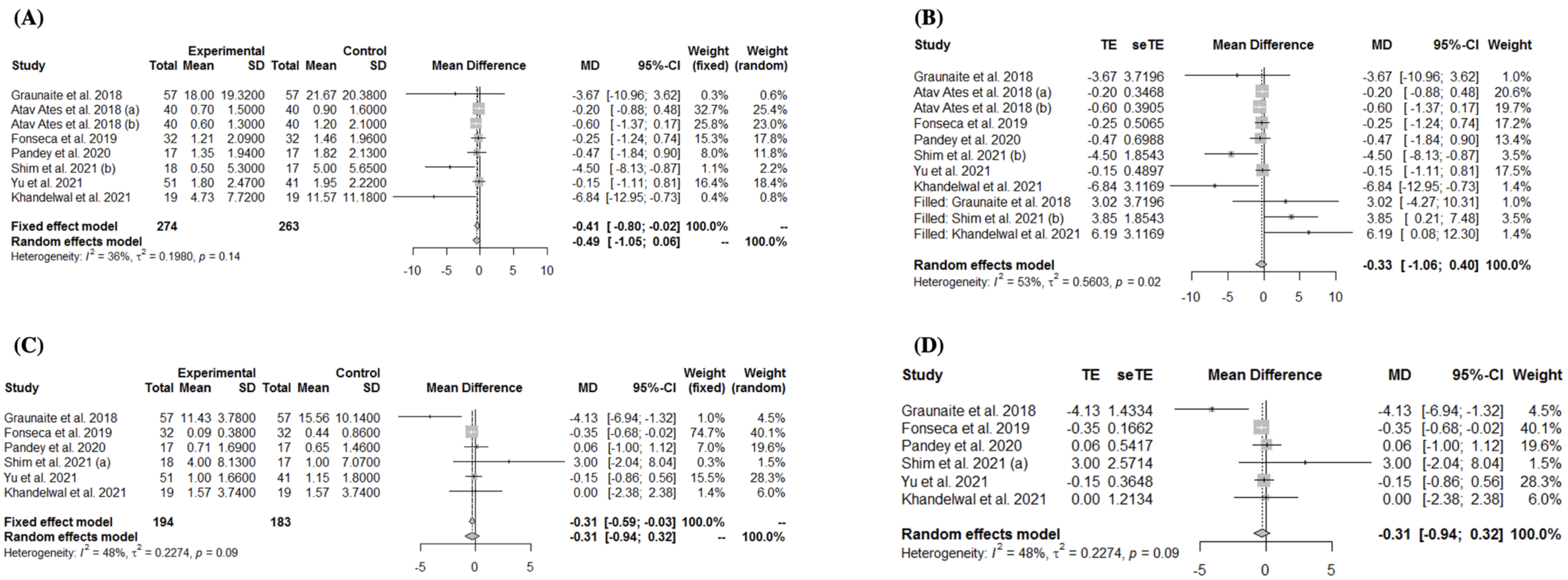
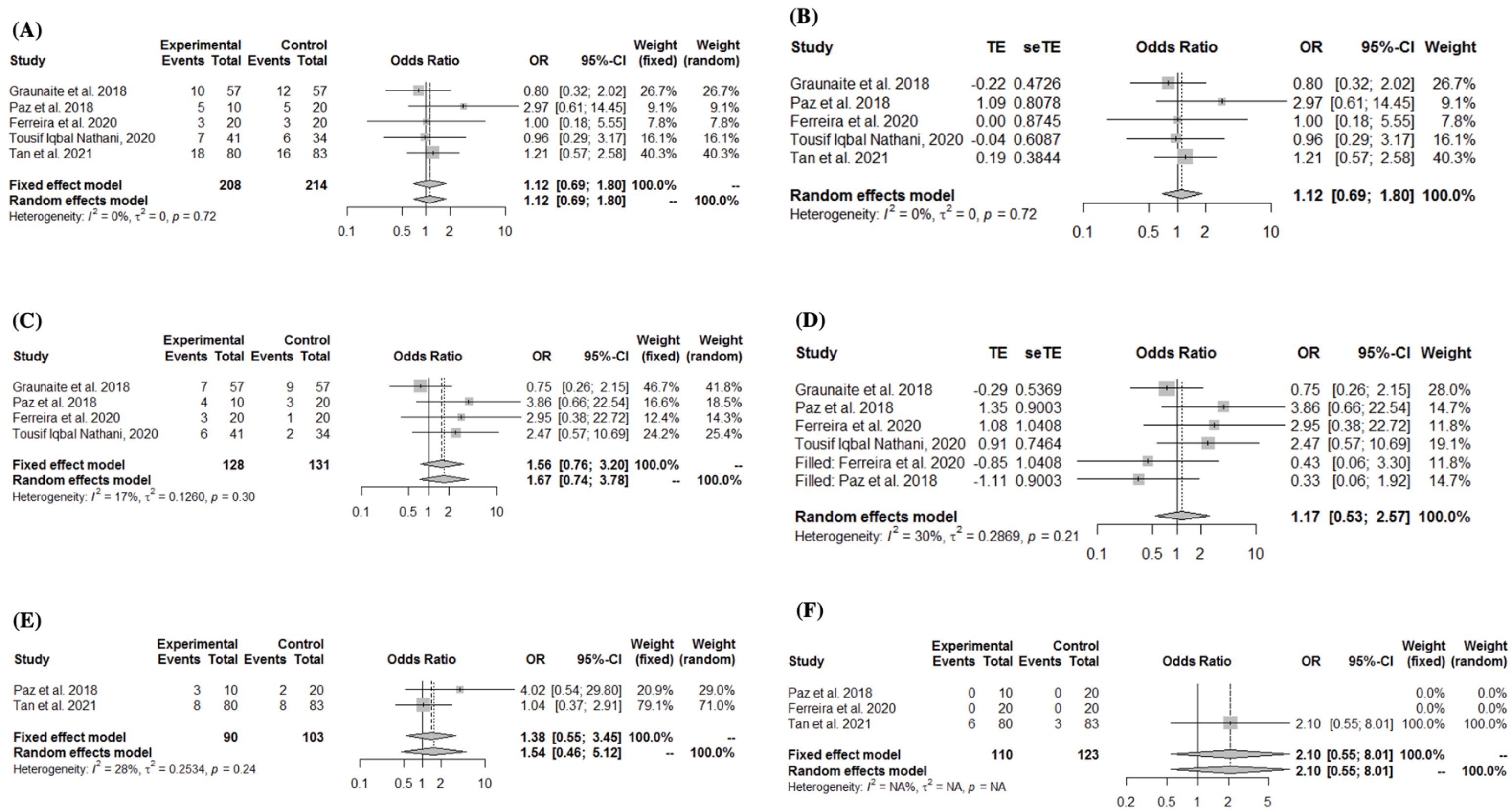


Figure 3. Meta-analysis binary variables for the periods of 24h, 48h, 72h and 7 days. A: Meta-analysis of 24h. B: Analysis of publication bias and meta-analysis of 24h (trim-and-fill). C: Meta-analysis of 48h. D: Analysis of publication bias and meta-analysis of 48h (trim-and-fill). E: Meta-analysis of 72h. F: Meta-analysis of 7 days



Chapter 2

*Standardization according to International Endodontic Journal Guidelines
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3 CHAPTER 2 - COMPARATIVE EVALUATION OF DENTINAL TUBULES PENETRATION AND ANTIMICROBIAL ACTIVITY OF BIOCERAMICS SEALERS AND AH PLUS AS ROOT CANAL FILLING MATERIALS - A SYSTEMATIC REVIEW AND META-ANALYSIS

3.1 ABSTRACT

Background: Bioceramic sealers are widely studied due to their histological benefits and potential properties such as penetration into dentinal tubules and antimicrobial effect, which are essential to ensure adequate root canal filling.

Objectives: The present study aimed to evaluate whether bioceramic sealers have better penetration capacity in dentinal tubules and antimicrobial activity when compared to AH Plus® sealer in *in vitro* experimental models.

Methods: This review followed the PRISMA checklist. Papers that evaluated the penetrating effect on dentinal tubules and the antimicrobial activity of bioceramic sealers (intervention) compared to AH Plus® (control) were analyzed. The search was performed in the following databases: PubMed, Scopus, Web of Science, Embase and Cochrane Library until August 20, 2021. with no restrictions on language or publication date. The search strategy was developed only with articles published in English language and with no publication time restrictions. Joanna Briggs qualifier was used to determine the risk of bias in the studies. Meta-analyses were performed by measuring the effect between standardized mean differences (SMD), followed by the inverse variance method, adopting a 95% confidence interval (CI).

Results: A total of 54 studies were included in this systematic review, and 16 studies were included in the meta-analysis. Overall, the papers presented low risk of bias. There was no statistical difference between the cements evaluated for penetration into dentinal tubules, regardless of the thirds evaluated: coronal SMD 0.58 [0.14; 1.31], $p = 0.12$; middle SMD 0.07 [0.54; 0.39], $p = 0.75$; and apical: SMD 0.08 [0.73; 0.56], $p = 0.80$. Bioceramic sealers and AH Plus® demonstrated similar antimicrobial action (SMD [3.42; 5.32], $p = 0.67$ and SMD 0.67 [1.89; 0.55], $p = 0.2825$, respectively).

Discussion: Possibly the meta-analyses showed similar effects between the sealers analyzed in both outcomes, mainly evaluated by the adequate properties of the sealants, since the bioceramics have smaller particles and the AH Plus® contains resin in its composition, which can enclose the microorganism. Even with distinct antimicrobial mechanisms, such as alkalinization capacity for bioceramics and release of toxic by-product for AH Plus®, both showed similar efficiency.

Conclusions: Bioceramic sealers and AH Plus® have similar penetration capacity in dentinal tubules and antimicrobial effect, making them suitable materials for clinical use.

Keywords: AH Plus; Antimicrobian activity; Endodontics; Penetration into dentinal tubules; Root canal sealer; Systematic Review; Calcium silicate; Meta-analysis.

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3.2 INTRODUCTION

Careful execution, appropriate choice of endodontic sealer, followed by the analysis of each step of root canal filling procedure are fundamental steps for the success of endodontic therapy, as this procedure aims to hermetically fill the root canal system avoiding the passage of fluids or microorganisms between the periodontium and the root canals. When properly performed, it ensures a better treatment prognosis (Ng *et al*. 2008, Kim *et al*. 2021). Thus, knowing the sealers used and knowing their behavior is an essential attribute for clinicians.

Among the sealers available, the AH Plus® endodontic sealer is an epoxy resin-based product that has stood out in recent years for presenting desirable physicochemical properties, such as good adhesion to root dentin, good flow capacity, apical sealing, radiopacity, solubility, long-term dimensional stability, and acceptable setting time, beeing considered a gold standard filling material (Duarte *et al*. 2010, Vertuan *et al*. 2018, Aksel *et al*. 2021). On the other hand, as it is an epoxy resin-based sealer, it is known that it does not have good biostimulating characteristics, such as inefficient induction of tissue mineralization, and some studies report satisfactory antimicrobial effect (Faria-Júnior *et al.* 2013, Viapiana *et al.* 2014, Oh *et al.* 2020). As a result, in recent years, bioceramic sealers have gained ground in the market and have aroused clinical interest, mainly due to the report of favorable biological effects.

Bioceramic sealers are characterized by being bioactive, bioinert and biodegradable, originating from bioceramic technology for medical and dental applications (Debelian & Trope 2016, Donnermeyer *et al.* 2019). In the formulation of these sealers, particles of alumina, zirconia, glass bioactives, calcium silicates, hydroxyapatite and calcium phosphates are present (Debelian & Trope 2016, Donnermeyer *et al.* 2019). During the sealer setting process, calcium hydroxide is released, which interacts with phosphates in tissue fluids and induces tissue mineralization through a chemical bond between dentin and sealer (Loushine *et al.* 2011, Debelian & Trope 2016). This interaction is intended to allow greater uniformity and prevent the formation of spaces in the filling, as well as to penetrate more efficiently into the root canal system (Mamootil & Messer 2007, Khalil *et al.* 2016). In addition, this group of cements has distinguished itself in its ability to promote good penetration into dentinal tubules, a characteristic that is extremely desired and required for these materials, as it can directly affect its antimicrobial effect. Since the greater the penetration power into the dentinal tubules, the better the retention and trapping of microbial residues (Mamootil & Messer 2007, Khalil *et al.* 2016). In addition to this, bioceramic sealers are able to maintain the local medium alkaline, therefore they may have potent antimicrobial activity inside the root canal (Loushine *et al.* 2011, Koutroulis *et al.* 2019).

Thus, due to the scarcity of systematic reviews that assess the potential of penetration into dentinal tubules and antimicrobial effect of bioceramic sealers, added to the lack of consensus on the most beneficial sealer in promoting such effects, the aim of this research was to evaluate through a systematic review and meta-analysis the penetration capacity into dentinal tubules and antimicrobial activity of bioceramic sealers compared to AH Plus® sealer.

3.3 MATERIAL AND METHODS

Protocol and registration

This systematic review was conducted under the guidance of the Cochrane Handbook for Systematic Reviews of Interventions (Higgins *et al.* 2019), following the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA)

(Liberati *et al*. 2009). The study registration protocol was submitted and registered in the Open Science Framework (OSF) Registries (https://doi.org/10.17605/OSF.IO/BX7VQ).

Search strategy

The electronic search was performed in the following databases: PubMed (MEDLINE), Scopus, Web of Science, Embase and Cochrane Library until August 20, 2021. In addition to performing some search in gray literature (produced at government, academic, business and industrial, in print or electronic format, but not controlled by commercial publishers) was searched using the OpenGrey database (http://www.opengrey.eu/ http://www.opengrey.eu/). The terms Mesh (Medical Subject Heading) were used to construct the search strategy (Table 1), established with the help of a specialized librarian, and to make the search more sensitive to the topic. Free terms were included that are not found as descriptors in the databases.

The search strategy was developed only with articles published in English language and with no publication time restrictions. A manual search was also performed to identify manuscripts that may not have been retrieved by electronic search, for articles published in the following journals: *Journal of Endodontics, International Endodontic Journal, Australian Endodontic Journal, Clinical Oral Investigations, Journal of Dentistry, European Endodontic Journal,* and *Iranian Endodontic Journal*. A manual screening of the reference lists of all included studies was performed so that no relevant articles were missed. Expert authors were contacted for information about ongoing studies and unpublished data.

Question PICO and eligibility criteria

A specific question was asked based on population, intervention, comparison and outcome (PICO): “Do bioceramic filling sealers have superior penetration into dentinal tubules and antimicrobial activity over AH Plus® filling sealer?”. Regarding the criteria, the population consisted of human and bovine teeth; the intervention was related to bioceramic sealers; the comparison was related to AH Plus® filling sealer; and the outcome evaluated was the ability to penetrate dentinal tubules and antimicrobial activity.

For this systematic review, *in vitro* studies that evaluated penetration into dentinal tubules and/or antimicrobial activity with bioceramic sealers (intervention) compared to AH Plus® sealer (comparison) were considered and included. Studies that did not standardize the filling protocol for groups, studies that included artificial teeth, conference abstracts, literature review articles, and studies based on research or expert opinion were excluded.

Studies selection

References recorded in all databases were imported into the online reference manager (EndNote Web; Thomson Reuters Inc., Philadelphia, PA, USA) and duplicates were excluded. Two reviewers (M.A.S. and G.P.N.) independently assessed all remaining titles and abstracts. In cases of titles and abstracts with insufficient information, the full texts were obtained for full reading. Any discrepancies were solved through a meeting and a third author (G.S.A.) was consulted when necessary. The agreement between the two reviewers regarding the title and selection of abstracts was assessed using Cohen's kappa coefficient ($\varkappa$). Subsequently, the full texts of the other articles were analyzed and those that meet the inclusion criteria were included.

Data extraction process

Details associated with the study were acquired using customized extraction forms, on which the following parameters were recorded: study details (authors and year of publication) and intervention groups. For data related to penetration studies in dentinal tubules, the type of tooth and root, irrigation used, which fluorophore and evaluation method, main results and conclusion were collected (Table 2). For data related to antimicrobial activity studies, type of tooth and root (when present), microbiological evaluation method and type of microorganism analyzed, main results and conclusions were collected (Table 3). When necessary, the authors were contacted by email to request additional information.

Risk of bias in included studies

Two authors (M.A.S., G.P.N.) independently assessed the risk of bias in the methodological quality of the selected studies, according to their levels of evidence, as proposed by the Checklist for Quasi-Experimental Studies (non-randomized experimental studies) scale of The Joanna Briggs Institute Critical Appraisal Tools for use in JBI Systematic Reviews (Tufanaru *et al*. 2017), with adaptations proposed by Silva *et al*. (2019) aiming to contemplate a better methodological evaluation of in vitro studies. Thus, the scales judged were as follows: clearly stated objective, justification of the sample size, randomization of samples, the possibility of comparison between control and experimental groups, and statistical analysis performed. Each scale of the included studies could be classified as "low, "high" or "unclear" risk of bias (Table 4). Doubts and discrepancies between the two evaluators were discussed and resolved by discussion and a third author (G.S.A.) when necessary.

Meta-analysis

The meta-analyses were performed using the R software with the "Meta" package, version 3.6.3, to evaluate bioceramic sealers versus AH Plus® sealer in terms of penetration capacity in dentinal tubules and antimicrobial activity. For the results, the effect measure was used between the standardized mean difference (SMD), and for the calculation of the meta-analysis, the inverse variance method was used. The meta-analyses were performed according to the apical root thirds for penetration into dentinal tubules (Coronal, Middle and Apical). Furthermore, the prediction range (PR) (for sample sizes close to or greater than 10) were expressed in the meta-analyses, representing a wider dispersion for an estimate of the range of expected real effects in future studies. In both analyses, the random effect model was applied with a 95% confidence interval (CI). Heterogeneity was tested using the I^2 index, and an I^2 index ≥ 50% was considered substantial or high. To check for publication bias, the trim-and-fill method and funnel plot symmetry visualization were used.

3.4 RESULTS

Literature Search

The flowchart of the literature search is shown in Figure 1. A total of 1486 documents were identified (PubMed: 916; Scopus: 251; Embase: 52; Cochrane: 22; Web of Science: 245; Open Gray: 00), of which 1114 remained for title and abstract screening after removing duplicates. The agreement between the two reviewers regarding the selection of titles and abstracts was $\varkappa$ = 0.915. From a total of 125 registries fully evaluated, 54 studies met pre-established eligibility criteria and were included in the systematic review (Fig. 1).

Characteristics of Included Studies

All 54 studies included in this systematic review were complete *in vitro* articles that addressed the outcomes related to penetration into dentinal tubules (n=27) (Table 2) and antimicrobial activity (n=27) (Table 3). Regarding the methods adopted to assess the ability of sealers to penetrate the dentinal tubules, 21 included studies used the Confocal Laser Scanning

Microscopy (CLSM) method (Kara Tuncer *et al.* 2014, Amoroso-Silva *et al.* 2014, Silva *et al.* 2015, Nikhil *et al.* 2015, Akcay *et al.* 2016, Menezes *et al.* 2017, Cruz *et al.* 2017, Piazza *et al.* 2017, Jardim Del Monaco *et al.* 2018, Kim *et al.* 2019, El Hachem *et al.* 2019, Giovarruscio *et al.* 2020, De Bem *et al.* 2020, Coronas *et al.* 2020, Yang *et al.* 2021, Muedra *et al.* 2021, Kakani *et al.* 2021, Furtado *et al.* 2021, Eskander *et al.* 2021, Alim Uysal *et al.* 2021), 4 studies used Scanning Electron Microscopy (SEM) (Sonu *et al.* 2016, Chen *et al.* 2017, Turkyilmaz & Erdemir 2020, Caceres *et al.* 2021) and only 1 study evaluated it by radiographic method (Candeiro *et al.* 2019) and Fluorescent microscopy (Khalil & Mahdee 2021). Of the studies that adopted the Confocal Laser Scanning Microscopy (CLSM) method, 18 studies used the fluorophore Rhodamine B (Kara Tuncer *et al.* 2014, Amoroso-Silva et *et al.* 2014, Silva *et al.* 2015, Nikhil *et al.* 2015, Akcay *et al.* 2016, Menezes *et al.* 2017, Cruz *et al.* 2017, Piazza *et al.* 2017, Jardim Del Monaco *et al.* 2018, Kim *et al.* 2019, Gunes *et al.* 2019, El Hachem *et al.* 2019, Giovarruscio *et al.* 2020, Yang *et al.* 2021, Muedra *et al.* 2021, Kakani *et al.* 2021, Eskander *et al.* 2021, Alim Uysal *et al.* 2021), one study used Fluo-3 (Coronas *et al.* 2020) and 2 studies used both fluorophores (De Bem *et al.* 2020, Furtado *et al.* 2021).

For microbiological quantification, most studies used the CFU/mL Count method (Ozcan *et al.* 2013, Faria-Júnior *et al.* 2013, Nirupama *et al.* 2014, Candeiro *et al.* 2016, Kapralos *et al.* 2018, Zordan-Bronzel *et al.* 2019, Šimundić Munitić *et al.* 2020, Bose *et al.* 2020, Barbosa *et al.* 2020, Allahem *et al.* 2020, Marashdeh *et al.* 2021, Kapralos *et al.* 2021, Carvalho *et al.* 2021 , Caldas *et al.* 2021), 7 studies used the CLSM method (Wang *et al.* 2014, Du *et al.* 2015, Arias-Moliz & Camilleri 2016, Bukhari & Karabucak 2019, Alsubait *et al.* 2019, Long *et al.* 2020 , Zancan *et al.* 2021), 5 studies used the halo of inhibition method (Weckwerth *et al.* 2015, Shakya *et al.* 2016, Dalmia *et al.* 2018, Pawińska *et al.* 2019, Rathod *et al.* 2020) and only a study of the SEM method (Willershausen *et al.* 2011). In general, a wide variety was observed in the species of microorganisms tested, with a majority predominance for the *Enterococcus faecalis* strains.

Risk of bias

The risk of bias analysis of the studies showed a low risk, as most of the questioned items were evaluated as "yes", which increased the reliability of the included studies. Notably, the question about the follow-up period was rated as "not applicable" to selected studies because all included studies were *in vitro*. In studies related to the outcome of penetration of dentinal tubules, it was observed that most studies did not justify the sample size, only 2 studies reported the same (Furtado *et al.* 2021, Yang et *et al.* 2021). 8 studies did not make explicit how the randomization of the samples was performed, thus they were considered as an "not clear" bias (Silva *et al.* 2015, Menezes *et al.* 2017, Kim *et al.* 2019, Gunes *et al.* 2019, Candeiro *et al.* 2019, Giovarruscio *et al.* 2020, Khalil & Mahdee 2021, Kakani *et al.* 2021). Overall, all studies that assessed antimicrobial activity were considered to be at "low risk" of bias. The risk of bias of the studies was compiled and is presented in Table 4.

Meta-analysis

In the meta-analyses performed for the outcome penetration into the dentinal tubules, there was no statistically significant differences between the bioceramic sealers and AH Plus®, regardless of the root thirds evaluated: coronal - SMD 0.5793 [-0.1495; 1.3082], p = 0.1193 (Fig. 2A); middle - SMD -0.0749 [-0.5438; 0.3941], p = 0.7543 (Fig. 2D); apical - SMD -0.0839 [-0.7319; 0.5640], p = 0.7996 (Fig. 2G). Two articles related to meta-analysis of the coronal third (Khalil & Mahdee 2021, Alim Uysal *et al.* 2021) and four to the apical third (Chen *et al.* 2017, Gunes *et al.* 2019, Khalil & Mahdee 2021, Eskander *et al.* 2021) demonstrated possible publication bias by the trim-and-fill method (Fig. 2B, 2E, and 2H) and funnel plot (Fig. 2C, 2F, and 2I).

Subgroup meta-analyses were carried out considering the root thirds at the expense of the types of bioceramic sealers used (MTA Fillapex and EndoSequence BC Sealer), regardless of the bioceramic sealer, all of them had a similar effect to AH Plus® in all root thirds analyzed: MTA Fillapex versus AH Plus®: coronal SMD 2.0506 [-0.0106; 4.1119], p = 0.0512 (Fig. 3A); middle SMD 0.6786 [-0.5104; 1.8676], p = 0.2633 (Fig. 3C); and apical SMD 0.6855 [-1.1945; 2.5654], p = 0.4748 (Fig. 3E). To verify publication bias, the trim-and-fill test was applied, two articles had possible failures for the coronal third (Sonu *et al.* 2016, Khalil & Mahdee 2021) (Fig. 3B), one article for the middle third (Sonu *et al.* 2016) (Fig. 3D) and two for the apical third (Sonu *et al.* 2016, Khalil & Mahdee 2021) (Fig. 3F). For the EndoSequence BC Sealer versus AH Plus® subgroup analysis: coronal SMD 0.0560 [-0.7495; 0.8615], p = 0.8916 (Fig. 4A); middle 0.0298 [-0.9690; 1.0286], p = 0.9534 (Fig. 4C); and apical SMD 0.3014 [-0.6884; 1.2911], p = 0.5506 (Fig. 4E). After applying the trim-and-fill method, it was observed that for the coronal third (Fig. 4B) and middle (Fig. 4D) no articles were found with publication bias, however, for the apical third, 1 article was found with possible publication bias (Eskander *et al.* 2021) (Fig. 4F).

For the antimicrobial activity outcome, two meta-analyses were performed for the bioceramic sealers, TotalFill BC Sealer and BioRoot RCS, compared to AH Plus® for *Enterococcus faecalis* strains. In both bioceramic sealers evaluated, there were no statistical differences to AH Plus® for antimicrobial activity: SMD [-3.4248; 5.3228], p = 0.6706 (Fig. 5A); and SMD -0.6692 [-1.8895; 0.5512], p = 0.2825 (Fig. 5C), respectively. Publication bias was not verified by the trim-and-fill method in any included study (Fig. 5B).

3.5 DISCUSSION

Penetration into dentinal tubules and antimicrobial activity are important properties for filling sealers, as these must fill the entire length of the root canal to form a solid, well-sealing filling (Grossman 1982). Since not all microorganisms are eliminated by biomechanical preparation or intracanal medication (Vera *et al.* 2012), these microorganisms can be trapped in the dentinal tubules by filling sealers (Siqueira & Rôças 2008, Ordinola-Zapata *et al.* 2009), highlighting the importance of evaluating these characteristics in root canal sealers. Thus, this systematic review and meta-analysis evaluated, in an *in vitro* model, the penetration into dentinal tubules and antimicrobial effect of bioceramic sealers (intervention) compared to AH Plus® sealer (control), and concluded that both sealers have similar penetration capacity in dentin tubules and antimicrobial action, regardless of the bioceramic sealer used (MTA Fillapex, EndoSequence BC Sealer, BioRoot RCS, and TotalFill BC Sealer).

It is important to point out that AH Plus® is currently considered the gold standard among filling sealers, as it presents satisfactory physical-chemical characteristics and properties, in addition to acceptable biocompatibility and bioactivity for the tissues, as well as good radiopacity, flow and setting time (Ferreira *et al.* 2021). Bioceramic sealers based on calcium silicates, calcium phosphate and calcium oxide, on the other hand, have been widely investigated in recent years, for their strong biomineralizing action promoted by the prolonged release of hydroxyl ions, in addition to triggering an excellent antimicrobial activity (Camilleri 2011, Wang *et al.* 2014, Khalil *et al.* 2016, Urban *et al.* 2018), and also be biocompatible with tissues (Rodríguez-Lozano *et al.* 2017, Zordan-Brozel *et al.* 2019). Based on these findings, the absence of statistical difference observed in all meta-analyses performed may be related to the excellent performance provided by both sealers, so that the satisfactory biological response inherent to their characteristics possibly provided similar behavior between them.

Quantitative synthesis related to microbiological evaluation was conducted on the antimicrobial effect of sealers on *E. faecalis*. It is noteworthy that this group of bacteria are gram-positive and anaerobic are related to failures of primary endodontic treatment, ranging in prevalence from 24% to 77% of secondary infections and have the ability to colonize dentinal tubules (Love 2001, Stuart *et al.* 2006, Siqueira & Rôças 2008, Ordinola-Zapata *et al.* 2009).

The similar antimicrobial activity of both sealers on the *E. faecalis* strain suggests that these materials can guarantee a promising effect on the success and longevity of endodontic treatments. Although the mechanisms of action that promote an antimicrobial effect are different among sealers, since bioceramics have alkalinization capacity; and the AH Plus® toxicity by the release of bisphenol A diglycidyl (Kapralos *et al.* 2021). Both are effective, as they demonstrated competent bactericidal capacity against this microorganism (Kapralos *et al.* 2021), evidenced by the intense reduction in the microbial load reported in the included studies, which suggests that both are indicated for clinical use.

Bioceramics can present particles smaller than 1 μm in diameter (McMichael *et al.* 2016) and the diameter of dentinal tubules can vary from 1 to 4 μm (Cleghorn *et al.* 2008), even so, no difference was noted in the penetrating effect in the tubules between the evaluated sealers. It is known that microorganisms present a strong pattern of colonization and can be smaller than 1 μm (Cleghorn *et al.* 2008) and reach between 100 and 1000 μm in depth in the dentinal tubules (Ordinola-Zapata *et al.* 2009). Thus, the penetrating effect of sealers is a precious characteristic, since these microorganisms can be trapped inside the tubules. There is evidence that bioceramic sealers (especially MTA Fillapex and EndoSequence BC Sealer) and AH Plus® have similar results for flow capacity and correspond to the ISO 6876/2012 standard (Candeiro *et al.* 2012, Ferreira *et al.* 2021), thus, the excellent flow capacity of these can explain the similar penetration potential. It is noteworthy that these characteristics are fundamental, as they make the filling present a better filling of the root canal system, benefiting clinical results.

Furthermore, the use of ethylene diamine tetracetic acid (EDTA) was unanimous in all studies that evaluated the penetrating effect of sealers on dentinal tubules. It is known that EDTA is the most used chelating agent in endodontics, a use attributed to its efficient ability to remove the smear layer (Pérez-Heredia *et al.* 2008, Zancan *et al.* 2021). Furthermore, this substance provides an increase in disinfection (Arias-Moliz & Camilleri 2016, Zancan *et al.* 2021), in addition to providing an increase in the penetration capacity of sealers in dentinal tubules (Haapasalo & Orstavik 1987, de Souza Matos *et al.* 2021, Eskander *et al.* 2021) and to favor the antimicrobial action of sealers (Zancan *et al.* 2021, Arias-Moliz & Camilleri 2016).

Regarding the assessment methods used, confocal laser scanning microscopy is a safe and valid method to assess penetration into dentinal tubules (Jeong *et al.* 2017, Donnermeyer *et al.* 2021), as it is not necessary to process the samples and /or evaluated endodontic sealer, thus avoiding procedures that cause its alterations, such as dissection or any type of deformation (Jeong *et al.* 2017). It is noteworthy that most studies included for this outcome adopted this method, except 6 studies that adopted other forms of assessment, such as scanning electron microscopy (SEM) (Sonu *et al.* 2016, Chen *et al.* 2017, Turkyilmaz & Erdemir 2020, Caceres *et al.* 2021), fluorescent microscopy (Khalil & Mahdee 2021) and radiographic (Candeiro *et al.* 2019). It is noteworthy that the SEM is limiting in relation to the depth of penetration promoted by the filling sealer, since this method consists of a superficial analysis of the tubule region (Donnermeyer *et al.* 2021), in addition to being subject to the formation of artifacts due to sample processing (De Deus *et al.* 2004). Therefore, they are often unable to present a detailed result of the samples, which makes the process of systematic analysis of the study difficult (Mamootil & Messer 2007).

All included studies used the fluorophore marker Rodomina B as an evaluation method to verify the penetration of endodontic sealers into dentinal tubules, except for three studies that adopted the Fluo-3 marker (Coronas *et al.* 2020, De Bem *et al.* 2020, Furtado *et al.* 2021). The type of fluorophoto can influence the analysis, as the chemical composition of the endodontic sealer reacts with the fluorophore and can result in a false-positive effect (Donnermeyer *et al.* 2021, Furtado *et al.* 2021). Rhodomin B, for example, is a hydrophilic marker with lower affinity for calcium ions, therefore caution/limitation is recommended in its use to evaluate bioceramic sealers (Donnermeyer *et al.* 2021), as it was observed that this fluorophore can trigger a leaching effect and enable the disaggregation of the sealer, so that it can emit fluorescence in moist dentinal regions and make the marking even in the absence of sealer (Joeng *et al.* 2017). Thus, Fluo-3, a marker that enables binding to calcium ions, is recommended as ideal for evaluating bioceramic sealers (Joeng *et al.* 2017). Based on this assumption, as some studies used Rhodomina B for both groups analyzed, it is believed that this may have influenced the results in favor of bioceramic sealers in these studies. However, it is important to highlight that the included studies that evaluated bioceramic sealers using Fluo-3 labeling also showed no statistical difference to AH Plus (Coronas *et al.* 2020, De Bem *et al.* 2020, Furtado *et al.* 2021).

It is important to mention that this systematic review presents some limitations. The studies that evaluated the antimicrobial activity of sealers present methodological variations, such as: form of cell culture, method of evaluation and contact time of microorganisms with the endodontic sealer. In addition, the studies that evaluated the outcome penetration into dentinal tubules, even with methodological standardization, in recent years, the type of fluorophore marker used for each investigated sealer was questioned. Thus, it reinforces the need for further studies, especially clinical trials evaluating the success rate of therapy with the materials evaluated, in order to confirm the results obtained in *in vitro* conditions.

3.6 CONCLUSION

Taking into account the data obtained and the limitations of the study, we can conclude that bioceramic sealers and AH Plus have similar effects of penetration into dentinal tubules and antimicrobial activity.

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Table 1. Search strategy

Search Strategy
#1 ((Epoxy Resin-Based root canal sealer) OR (Root Canal Filling Materials) OR (Root Canal Filling Material) OR (Root Canal Sealants) OR (Root Canal Sealant) OR (Sealants, Root Canal) OR (Canal Sealant, Root) OR (Canal Sealants, Root) OR (Sealant, Root Canal) OR (AH Plus) OR (Adseal) OR (Epoxy Resin-Based Sealer) OR (Root Canal Filling) OR (AH Plus Sealer) OR (AH Plus Jet) OR (Resin-based sealer) OR (Endodontic Sealers)
#2 (Bioceramic) OR (Bioceramic Sealer) OR (Hydraulic Sealer) OR (Hydraulic Endodontic Sealer) OR (Hydraulic Calcium Silicate Sealers) OR (Hydraulic Sealer Cements) OR (Calcium Silicate-Based Sealers) OR (Calcium Silicate-Based Sealer) OR (Calcium Silicate-Root Canals Sealers) OR (Calcium Silicate-Root Canals Sealer) OR (EndoSequence BC Sealer) OR (Endoseal MTA) OR (TotalFill BC) OR (MTA Fillapex) OR (Sealer Plus BC) OR (iRoot SP) OR (BioRoot RCS) OR (Bio-C Sealer)
#3 (Dentinal Tubule) OR (Depth of Penetration) OR (Penetration Depth) OR (Sealer Penetration) OR (Sealing) OR (Tubule Penetration) OR (Antimicrobial Activity) OR (Antimicrobial Effect) OR (Antimicrobial Effectiveness) OR (Antibacterial Activity) OR (Antibacterial Affect) OR (Antibacterial Effectiveness) OR (Anti-biofilm Activity) OR (Anti-biofilm Affectiveness)
#1 AND #2 AND #3

Table 2. General data from selected studies of penetration into dentinal tubules

Author/ year	G1 - AH Plus group (control group) G2 - Bioceramic group (N sample)	Tooth root and tooth type	Final irrigation protocol	Method	Applied dye	Sealer tubular penetration [Mean ± SD] µm or percentage	Conclusion
Alim Uysal *et al*. 2021	G1 (AH Plus): 7 G2.1 (MTA Fillapex): 7 G2.2 (EndoSequence BC Sealer): 7	Mandibular premolar teeth Human	Saline (control): 2mL 2.5% NaOCl + saline EDTA group: 2mL 2.5% NaOCl + 17% EDTA for 1min Maleic acid group: 2mL 2.5% NaOCl + 7% Maleic acid for 1min Etidronic acid group: 2mL 2.5% NaOCl + 9% Etidronic acid	Confocal Laser Scanning Microscopy (CLSM)	Rhodamine B	G1 Apical: 444.40 ± 142.09 Middle: 705.65 ± 107.96 Coronal: 977.35 ± 228.67 G2.1 Apical: 457.60 ± 141.04 Middle: 726.50 ± 111.66 Coronal: 1007 ± 196.64 G2.2 Apical: 469.80 ± 93.39 Middle: 848.50 ± 140.95 Coronal: 1092 ± 198.23	EndoSequence BC Sealer displayed greater penetration depth than AH Plus and MTA Fillapex.
Caceres *et al*. 2021	G1 (AH Plus): 14 G2 (Bio-C Sealer): 14	Palatal roots of maxillary molars, distal roots of mandibular molars and roots of maxillary incisors Human	10 mL of NaOCl 2.5% 5 ml of EDTA 17% 5 ml of NaOCl 2.5% for a final rinse	Scanning Electron Microscope (SEM)	NR	G1 / G2 Apical: 0.17 ± 0.1 / 0.25 ± 0.11 Middle: 0.70 ± 0.56 / 1.87 ± 2.58 Coronal: 0.81 ± 0.8 / 1.97 ± 1.73	Bio-C Sealer presents greater penetration and better tubular adaptation than AH-Plus.
Eskander *et al*. 2021	G1 (AH Plus): 10 G2 (EndoSequence BC Sealer): 10	Mandibular single-rooted premolar teeth Human	2 ml of 2.5% NaOCl 1 min final irrigation with 17% EDTA	Confocal Laser Scanning Microscopy (CLSM)	Rhodamine B	G1 / G2 Apical: 284.39 ± 120.47 / 1.09 ± 0.67 Middle: 444.50 ± 112.59 / 2 ± 0.85 Coronal: 324.15 ± 201.35 / 1.09 ± 17.21	The increase in tubular penetration was detected in the AH Plus sealer group.

Furtado *et al*. 2021	G1 (AH Plus): 20 G2.1 (Sealer Plus BC): 20 G2.2 (EndoSequence BC Sealer): 20	Mandibular teeth Bovine	2 ml of 2.5% NaOCl Rinsed with 0.9% saline solution 2 ml of 17% EDTA (agitated for 3 mim) Final rinse with 2 ml of 0.9% saline solution	Confocal Laser Scanning Microscopy (CLSM)	Rhodamine B and Fluo-3	G1 0.1% Rhodamine B Apical: 85.49 ± 15.05 Middle: 88.97 ± 12.27 Coronal: 90.25 ± 14.74 Fluo-3 Apical: 66.97 ± 15.38 Middle: 76.59 ± 10.85 Coronal: 78.80 ± 10.75 G2.1 0.1% Rhodamine B Apical: 92.52 ± 4.87 Middle: 91.80 ± 3.50 Coronal: 91.67 ± 7.38 Fluo-3 Apical: 67.46 ± 14.29 Middle: 80.42 ± 11.31 Coronal:78.96 ± 14.53 G2.2 0.1% Rhodamine B Apical: 93.59 ± 10.19 Middle: 92.06 ± 9.66 Coronal: 89.68 ± 7.66 Fluo-3 Apical: 69.77 ± 7.60 Middle: 78.75 ± 8.88 Coronal: 73.72 ± 8.45	The Sealer Plus BC and EndoSequence BC Sealer cements showed more favorable results.
Kakani *et al*. 2021	G1 (AH Plus): 10 G2 (Smartpaste Bio): 10	Single-rooted mandibular premolars Human	2.5% NaOCl 17% EDTA Distilled water rinse	Confocal Laser Scanning Microscopy (CLSM)	Rhodamine B	Mean depths of sealer penetration of all the groups at various root regions G1: 153.4490 ± 9.39737 G2: 232.4690 ± 9.97453 **Percentage** G1 / G2 Apical: 369.99 / 301.81 Middle: 947.57 / 937.85 Coronal: 1217.91 / 1148.32	No differences were observed in the penetration of dentinal tubules for AH Plus and bioceramics.

Khalil & Mahdee 2021	G1 (AH Plus): 5 G2.1 (MTA Fillapex): 5 G2.2 (TotalFill BC Sealer): 5	Mandibular first premolar Human	NR	Fluorescent microscopy	NR	G1 Apical: 0.23 ± 0.045 Middle: 0.298 ± 0.047 Coronal: 0.432 ± 0.004 G2.1 Apical:0.218 ± 0.044 Middle: 0.276 ± 0.024 Coronal: 0.36 ± 0.023 G2.2 Apical: 0.218 ± 0.034 Middle: 0.322 ± 0.019 Coronal: 0.39 ± 0.029	Root canal sealer have a similar effect for penetration into dentinal tubules.
Muedra *et al*. 2021	G1 (AH Plus): 20 G2.1 (EndoSequence BC Sealer): 20 G2.2 (BioRoot RCS): 20	Maxillary and mandibular single-rooted teeth Human	3 mL of 5.25% NaOCl 17% EDTA during 1 min 5 mL saline solution	Confocal Laser Scanning Microscopy (CLSM)	Rhodamine B	G1 Apical: 193.25 ± 431.55 Middle: 449.03 ± 612.64 Coronal:1118.01 ± 727.53 G2.1 Apical: 475.35 ± 404.81 Middle: 981.33 ± 581.14 Coronal: 1316.57 ± 804.57 G1.2 Apical:304.32 ± 505.02 Middle: 508.11 ± 715.84 Coronal: 743.74 ± 781.18	EndoSequence BC Sealer exhibited the highest tubular penetration, while no significant differences were found between BioRoot RCS and AH Plus.
Yang *et al*. 2021	G1 (AH Plus): 13 G2.1 (iRoot SP): 26 G2.2 (EndoSequence BC Sealer HiFlow): 26	Single-rooted premolars Human	2 mL 3% NaOCl 10 mL 17% EDTA for 1min Final flush with saline solution	Confocal Laser Scanning Microscopy (CLSM)	Rhodamine B	**Percentage** G1 Continuous wave condensation 4 mm (Apical): 15% 8 mm (Middle): 20% 12 mm (Coronal): 19% G2.1 Continuous wave condensation 4 mm (Apical): 12% 8 mm (Middle): 22% 12 mm (Coronal): 22% Single-cone 4 mm (Apical): 8%	EndoSequence BC Sealer showed better performance in dentinal tubule penetration than iRoot SP.

						8 mm (Middle): 18% 12 mm (Coronal): 10% G2.2 Continuous wave condensation 4 mm (Apical): 26% 8 mm (Middle): 28% 12 mm (Coronal): 24% Single-cone 4 mm (Apical): 14% 8 mm (Middle): 16% 12 mm (Coronal): 9%	
Coronas *et al*. 2020	G1 (AH Plus): 10 G2 (Sealer Plus BC): 20	Superior molars teeth with three distinct roots Human	2 mL of 2,5% NaOCl (syringe) 5 mL of 2.5% NaOCl (passive ultrasonic irrigation) 2 mL of 17% EDTA Rinse with saline solution	Confocal Laser Scanning Microscopy (CLSM)	Fluo-3	G1 Lentulo 2 mm (Apical): 2.91 25^{th} percentile: 1.97 75^{th} percentile: 5.56 7 mm (Middle): 3.77 25^{th} percentile: 3.35 75^{th} percentile: 9.05 G2 Lentulo 2 mm (Apical): 7.92 25^{th} percentile: 6.59 75^{th} percentile: 9.55 7 mm (Middle): 8.46 25^{th} percentile: 4.77 75^{th} percentile: 10.25 G2 Ultrassonic insert 2 mm (Apical): 4.14 25^{th} percentile: 3.87 75^{th} percentile: 4.57 7 mm (Middle): 10.85 25^{th} percentile: 9.67 75^{th} percentile: 11.62	No differences were observed when Bioceramic Ah Plus groups, regardless the root third and method used evaluated.

De Bem *et al*. 2020	G1 (AH Plus): 20 G2.1 (MTA Fillapex): 20 G2.2 (Sealer Plus BC): 20 G2.3 (EndoSequence BC Sealer): 20	Teeth single-rooted with a single straight canal Human	20 mL 2.5% NaOCl 5 mL EDTA 17% for 5 min 10 mL distilled water	Confocal Laser Scanning Microscopy (CLSM)	Rhodamine B and Fluo-3	**No ultrasonic activation** G1 (rhodamine B) Apical: 12.15 ± 5.17 Middle: 18.54 ± 4.91 Mean: 15.34 ± 5.26 G2.1 (rhodamine B) Apical: 19.63 ± 7.43 Middle: 19.96 ± 7.93 Mean: 19.79±7.63 G2.2 (Fluo-3) Apical: 7.51 ± 3.66 Middle: 20.57 ± 7.75 Mean: 15.56 ± 5.41 G2.3 (Fluo-3) Apical: 10.83 ± 3.39 Middle: 12.13 ± 4.40 Mean: 11.48 ± 3.70 **With ultrasonic activation** G1 (rhodamine B) Apical: 15.20 ± 5.98 Middle: 26.57 ± 8.37 Mean: 20.88 ± 7.44 G2.1 (rhodamine B) Apical: 44.23 ± 10.23 Middle: 48.64 ± 8.10 Mean:42.55 ± 9.71 G2.2 (Fluo-3) Apical: 11.47 ± 4.30 Middle: 19.84 ± 7.57 Mean: 15.65 ± 6.96 G2.3 (Fluo-3) Apical: 21.20 ± 8.45 Middle: 20.61 ± 8.70 Mean: 25.20 ± 8.06	Conclude that ultrasonic activation positively interferes in the intratubular penetration of the endodontic sealers but in a significant way only for MTA Fillapex and EndoSequence BC Sealer.
	G1.1 (AH Plus + gutta-percha point and		5 mL of 1% NaOCl 2 mL of 17% EDTA		Rhodamine B	G1.1 Apical: 435.88 ± 293.95 Middle: 715.75 ± 222.41 Middle + Apical: 589.41 ± 296.18	The EndoSequence BC Sealer showed the deepest penetration of the sealer AH Plus.

Giovarruscio *et al*. 2020	warm vertical condensation): 20 G1.2 (AH Plus + CPoint): 20 G2 (EndoSequence BC Sealer): 20	Single rooted Human	Final rinse with NaOCl	Confocal Laser Scanning Microscopy (CLSM)		G1.2 Apical:176.06 ± 150.07 Middle: 463.31 ± 290.84 Middle + Apical: 324.16 ± 266.10 G2 Apical: 506.44 ± 326.19 Middle: 913.25 ± 212.55 Middle + Apical: 709.84 ± 340.67	
Turkyilmaz & Erdemir 2020	G1 (AH Plus): 20 G2 (MTA Fillapex): 20	Teeth with single-rooted canal Human	5 mL 5% NaOCl 5 mL 17% EDTA Rinsed with 5 ml distilled water	Scanning electron microscope (SEM)	NR	G1 Cold lateral condensation technique Mean total: 1286 ± 529 Max. depth Apical: 764 ± 286 Middle: 1312 ± 373 Coronal: 1745 ± 372 Min. depth Apical: 28 ± 17 Middle: 24 ± 14 Coronal: 19 ± 7 Single cone technique Mean total: 1458 ± 478 Max. depth Apical: 1154 ± 379 Middle: 1505 ± 399 Coronal: 1715 ± 505 Min. depth Apical: 45 ± 29 Middle: 53 ± 35 Coronal: 66 ± 45 G2 Cold lateral condensation technique Mean total: 1323 ± 497 Max. depth Apical: 969 ± 406 Middle: 1229 ± 366 Coronal: 1771 ± 304 Min. depth Apical: 45 ± 11	MTA Fillapex showed similar results against AH Plus.

						Middle: 33 ± 11 Coronal: 27 ± 11 Single cone technique Mean total: 1483 ± 528 Max. depth Apical: 973 ± 312 Middle: 1545 ± 322 Coronal: 1931 ± 364 Min. depth Apical: 39 ± 14 Middle: 32 ± 15 Coronal: 43 ± 25	
Candeiro *et al*. 2019	G1 (AH Plus): 13 G2 (Endosequence BC Sealer): 13	Maxillary premolars Human	40 mL of 5.25% NaOCl 5 mL of saline passive ultrasonic irrigation (PUI) with EDTA 17%	(Lateral canals were fabricated with distance of 2, 4, and 6 mm from apical foramen) Radiographic analysis (Score 0 – 4)	NR	Score G1 / G2 Apical: 2.58 ± 3 / 2.56 ± 3 Middle: 2.81 ± 3 / 2.63 ± 4 Coronal: 2.63 ± 3 / 2.98 ± 3	Penetration of bioceramic and epoxy-resin endodontic cements into lateral canals Endosequence BC Sealer presented lateral canal filling capacity similar to that of the epoxy-resin AH Plus in simulated canals.
El Hachem *et al*. 2019	G1 (AH Plus): 32 G2.1 (Endosequence BC Sealer): 32 G2.2 (novel tricalcium silicate sealer): 32	Maxillary central incisors Human	3 mL 5.25% NaOCl for 3 min 10 mL 17% EDTA 10 mL deionised water	Confocal laser scanning microscopy (CLSM)	Rhodamine B	G1: 1mm (Apical): 568.96 ± 222.89 5 mm (Middle): 984.29 ± 271.55 G2.1 1mm (Apical): 600.84 ± 235.935 5 mm (Middle): 1152.81 ± 257.51 G2.2 1mm (Apical): 656.89 ± 272.93 5 mm (Middle): 1063.61 ± 293.52	EndoSequence BC Sealer and NTS demonstrated better dentinal tubule penetration results compared to AH Plus.

Gunes *et al*. 2019	G1 (AH Plus): 10 G2 (Endosequence BC Sealer): 10	Mandibular premolar teeth Human	3 mL 5% NaOCl 3 mL 17% EDTA 3 mL distilled water	Confocal laser scanning microscopy (CLSM)	Rhodamine B	G1 / G2 Apical: 848.16 ± 162.38 / 728.07 ± 173.15 Middle: 1,503.05 ± 117.74 / 1,373.03 ± 150.37 Coronal: 1,409.27 ± 175.63 / 1,540.66 ± 85.81	No differences were observed when Bioceramic and AH Plus groups.
Kim *et al*. 2019	G1 (AH Plus): 20 G2.1 (BioRoot RCS): 20 G2.2 (MTA Endoseal): 20	Human premolars	2 mL of 2.5% NaOCl 2 mL of 17% EDTA 10 mL of distilled water	Confocal laser scanning microscopy (CLSM)	Rhodamine B	Maximum sealer penetration depth G1 Apical: 1035.73 Middle: 1291.41 Coronal: 1542.27 G2.1 Apical: 325.82 Middle: 1783.41 Coronal: 1132.02 G2.2 Apical: 455.13 Middle: 551.19 Coronal: 687.72	The maximum sealer penetration distance was higher at the apical third in the AH Plus group compared with BioRoot RCS and Endoseal MTA, and similar sealer penetration was observed at the middle and coronal third when comparing between AH Plus and BioRoot RCS.
Jardim Del Monaco *et al*. 2018	G1 (AH Plus): 10 G2 (Endosequence BC Sealer): 10	Mandibular uniradicular premolars Human	5 mL of 17% EDTA with passive ultrasonic irrigation (PUI) for 1 min 5 mL of 2.5% NaOCl	Confocal laser scanning microscopy (CLSM)	Rhodamine B	G1 / G2 Apical: 333 ± 197 / 473 ± 170 Middle: 415 ± 152 / 675 ± 249	The bioceramic sealer showed a higher penetration than the AH Plus® sealer group.
Piazza *et al*. 2017	G1 (AH Plus): 15 G2 (MTA Fillapex): 15	Incisor teeth Bovine	2 mL 2.5% NaOCl 5 mL 17% EDTA Saline solution	Confocal laser scanning microscopy (CLSM)	Rhodamine B	G1 / G2 Apical: 487.3 (0.0-1258) / 927(0.0-1463) Middle: 963.9 (474.7-1313) / 970.1 (0.0-1193) Coronal: 986.6 (761.6-1389) / 1050 (0.0-1215)	MTA Fillapex exhibited higher values of intratubular penetration when compared to AH Plus.

Chen *et al*. 2017	G1 (AH Plus): 12 G2 (iRoot SP): 12	Mandibular second premolars Human	4 mL of 2.5% NaOCl 3 mL 17% EDTA 5 mL of distilled water	Scanning Electron Microscopy (SEM)	NR	G1 / G2 Apical: 19.10 ± 7.87 / 6.78 ± 3.00 Middle: 26.13 ± 11.81 / 25.68 ± 11.01 Coronal: 69.82 ± 21.79 / 66.30 ± 24.46	The iRoot SP exhibited similar intratubular penetration values when compared to the AH Plus.
Cruz *et al*. 2017	G1 (AH Plus): 18 G2 (MTA Fillapex): 18	Mandibular premolars Human	5 mL of 2.5% NaOCl 3 mL of 17% EDTA	Confocal laser scanning microscopy (CLSM)	Rhodamine B	G1 / G2 Apical: 19.38 ± 10.35 / 55.76 ± 22.01 Middle: 76.86 ± 21.86 / 81.61 ± 15.66	MTA Fillapex obturations presented a higher tubular penetration than AH Plus obturations.
Menezes *et al*. 2017	G1 (AH Plus): 10 G2 (MTA Fillapex): 10	Single-rooted premolar teeth Humana	1 mL of 5.25% NaOCl 3 mL of 17% EDTA 5 mL of distilled water	Confocal laser scanning microscopy (CLSM)	Rhodamine B	Only Mean G1 / G2 Apical: 357.23 / 662.23 Middle: 574.44 / 937.72 Coronal: 750.78 / 868.09	MTA Fillapex had a greater ability to penetrate the dentinal tubules.
Akcay *et al*. 2016	G1 (AH Plus) 13 G2.1 (iRoot SP): 13 G2.2 (MTA Fillapex): 13	Mandibular premolars Human	5 ml of 5% NaOCl for 1 min 5 ml of 17% EDTA for 1 min	Confocal laser scanning microscopy (CLSM)	Rhodamine B	Area (mm^2) G1 Apical: 0.2 Middle: 0.3 Coronal: 0.47 G2.1 Apical: 0.14 Middle: 0.35 Coronal: 0.4 G2.2 Apical: 0.09 Middle: 0.24 Coronal: 0.29	Both root canal sealer had similar results for penetration into dentinal tubules.

Sonu *et al*. 2016	G1 (AH Plus): 10 G2 (MTA Fillapex) 10	Single-rooted mandibular premolar Human	3 ml of 5.25% NaOCl for 1 min 2 ml of 17% EDTA for 3 min Distiller water for 1 min	Scanning electron microscopic (SEM)	NR	G1 / G2 Apical: 47.3 ± 1.10353 / 19.48 ± 1.93953 Middle: 130.8 ± 10.7579 / 91.88 ± 3.1485 Coronal:122.3 ± 8.73117 / 81.5 ± 7.573198	AH Plus showed the maximum sealer penetration depth.
Nikhil *et al*. 2015	G1 (AH Plus): 30 G2 (MTA Fillapex): 30	Maxillary central incisors Human	2 mL of 3% NaOCl 2 mL of 17% EDTA 2 mL distilled water	Confocal laser scanning microscopy (CLSM)	Rhodamine B	G1 / G2 Ultrasonic Apical: 510.0 ± 31.3 / 664.0 ± 40.1 Coronal: 633 ± 48.5 / 788.0 ± 23.0 Lentulo Apical: 321.0 ± 34.1 / 411.0 ± 39.3 Coronal: 449.0 ± 47.5 / 545.0 ± 43.3 Counter-clockwise motion Apical: 292.0 ± 31.2 / 397.0 ± 43.5 Coronal: 404.0 ± 39.5 / 524.0 ± 45.8	MTA Fillapex sealer exhibited better percentage and depth of penetration in the radicular dentinal tubules than AH Plus.
Silva *et al*., 2015	G1 (AH Plus): 10 G2 (MTA Fillapex): 10	Single-rooted premolars Human	2 mL of 17% EDTA passive ultrasonic irrigation (PUI) for 3 min 1 mL of 5.25% NaOCl	Confocal laser scanning microscopy (CLSM)	Rhodamine B	G1 / G2 Apical: 83.07 ± 50.79 / 99.22 ± 59.38 Middle: 139.9 ± 70.19 / 157.0 ± 50.87 Coronal: 196.4 ± 79.54 / 163.6 ± 40.64	No differences were observed when MTA Fillapex and AH Plus groups.
Amoroso-Silva *et al*. 2014	G1 (AH Plus): 15 G2 (MTA Fillapex): 15	Maxillary central incisors Human	1 mL of 2.5% of NaOCl 3 mL of 17% EDTA 3 mL of distilled water	Confocal laser scanning microscopy (CLSM)	Rhodamine B	**Percentage** G1 / G2 Apical: 6% / 3% Middle: 62% / 42% Coronal: 65% / 65%	The MTA Fillapex showed intratubular penetration similar to the AH Plus.

Kara Tuncer *et al*. 2014	G1 (AH Plus): 10 G2.1 (iRoot SP): 10 G2.2 (MTA Fillapex): 10	Maxillary incisors with single canals Human	5 mL of 2.5% NaOCl 5 mL of 17% EDTA 5 mL of distilled water	Confocal laser scanning microscopy (CLSM)	Rhodamine B	**Percentage** G1 Apical: 30.16 ± 10.47 Middle: 37.71 ± 12.71 Coronal: 56.39 ± 19.93 G2.1 Apical: 22.15 ± 6.46 Middle: 27.09 ± 8.21 Coronal: 38.20 ± 13.31 G2.2 Apical: 32.82 ± 18.17 Middle: 48.12 ± 21.50 Coronal: 59.78 ± 18.03	AH Plus and MTA Fillapex showed significantly higher sealer penetration than those treated with iRoot SP.

Abbreviations: NaOCl = sodium hypochlorite; EDTA = ethylene diamine tetracetic acid; NR = not reported.

Table 3. General data from selected studies of antimicrobial activity.

Author/ year	G1 - AH Plus group G2 - Bioceramic group (N sample)	Tooth root and Tooth type	Type of microbiota	Microbiological assessment method	Microbial culture	Conclusion
Caldas *et al*. 2021	G1 (AH Plus) G2 (MTA Fillapex)	NR	*E. faecalis* (ATCC 29212)	CFU/mL Count	G1 / G2 20 min: 1.25 / 0.75 7 days: 6 / 4	The antimicrobial activity of AH Plus was similar to that of MTA Fillapex in freshly mixed conditions.
Carvalho *et al*. 2021	G1 (AH Plus) G2 (MTA Fillapex)	NR	*E. faecalis* (ATCC 29212)	CFU/mL Count	G1 / G2 Fresh samples 10 min: 0 / 4.91 ± 0.08 60 min: 0 / 4.89 ± 0.06 7 days 10 min: 4.32 ± 0.19 / 4.87 ± 0.04 60 min: 4.41 ± 0.03 / 4.80 ± 0.12 30 days 10 min: 4.66 ± 0.05 / 4.81 ± 0.04 60 min: 4.79 ± 0.04 / 4.88 ± 0.03	The bioceramic showed similarity to the AH plus sealer for antimicrobial activity.
Kapralos *et al*. 2021	G1 (AH Plus) G2 (BioRoot RCS)	NR	*E. faecalis* (ATCC) 19434, *S. mutans* (ATCC 700610), *S. epidermidis* (ATCC 35984) and *S. aureus*	CFU/mL Count	Planktonic forms G1 / G2 *E. faecalis* No saline: 7.591 ± 0.062 / 4.041 ± 0.440 Saline: 5.072 ± 0.671 / 5.227 ± 0.981 *S.mutans* No saline: 6.158 ± 0.962 / 0 Saline: 5.336 ± 0.628 / 5.47 ± 0.234 *S. epidermidis* No saline: 7.635 ± 0.143 / 5.447 ± 0.141 Saline: 5.403 ± 0.511 / 5.563 ± 0.652 *S. aureus* No saline: 6.547 ± 0.341 / 5.964 ± 0.688 Saline: 5.558 ± 0.978 / 4.924 ± 0.095	The bioceramics showed better effects for antimicrobial activity.

					Monospecies biofilm G1 / G2 *E. faecalis*: 10.510 ± 0.279 / 8.916 ± 1.306 *S. mutans*: 9.960 ± 0.234 / 8.472 ± 1.077 *S. epidermidis*: 10.599 ± 0.329 / 6.477 ± 0.323 *S. aureus*: 10.748 ± 0.202 / 8.346 ± 0.534	
Marashdeh *et al*. 2021	G1 (AH Plus) G2 (EndoSequence BC Sealer)	NR	*E. faecalis* (ATCC 47077)	CFU/mL Count	Planktonic *E. faecalis* G1 / G2 7 days: 6.125 / 4.14 ± 0.09 30 days:6.062 / 5.12 ± 0,1	The bioceramic sealer showed antimicrobial activity.
Zancan *et al*. 2021	G1 (AH Plus): 5 G2.1 (BioRoot RCS): 5 G2.2 (MTA Fillapex): 5 G2.3 (TotalFill BC Sealer): 5	Maxillary incisors Human	*E. faecalis* (ATCC 29212)	Confocal Laser Scanning Microscopy (CLSM) Protocol A 2% NaOCl – 5 mL/5 min Protocol B 2% NaOCl – 5 mL/5 min 17% EDTA – 5 mL/3 min.	% of live cells Protocol A G1: 81.63 % (63.99 – 96.42) G2.1: 70.09 % (59.23 – 82.53) G2.2: 77.43 % (50.74 – 98.46) G2.3: 88.06 % (54.23 – 96.96) Protocol B G1: 76.18 % (50.02 – 98.26) G2.1: 53.06 % (15.82 – 73.00) G2.2: 67.00 % (50.93 – 92.21) G2.3: 76.92 (61.54 – 94.89)	BioRoot was a more effective sealer in reducing the bacterial load in the dentinal tubules than the other materials tested.
Allahem *et al*. 2020	G1 (AH Plus): 4 G2.1 (MTA Fillapex): 4 G2.2 (EndoSequence BC Sealer): 4	NR	*E. faecalis*, *P. intermedia*, *F. nucleatum* and *P. micra*	CFU/mL Count	Amount of viable bacteria (mean ± SD) G1 24h *E. faecalis*: 3.61 ± 0.22 *F. nucleatum*: 0.00 ± 0 *P. intermedia*: 0.00 ± 0 *P. micra*: 0.00 ± 0 48h *E. faecalis*: 4.00 ± 0 *F. nucleatum*: 0.00 ± 0 *P. intermedia*: 0.00 ± 0 *P. micra*: 0.00 ± 0 5 days *E. faecalis*: 4.44 ± 0.46	AH Plus sealer showed the highest antibacterial, while MTA Fillapex sealer

					F. nucleatum: 0.00 ± 0 *P. intermedia*: 0.00 ± 0 *P. micra*: 0.00 ± 0 7 days *E. faecalis*: 4.56 ± 0.46 *F. nucleatum*: 0.00 ± 0 *P. intermedia*: 0.00 ± 0 *P. micra*: 0.00 ± 0 G2.1 24h *E. faecalis*: 0.56 ± 0.52 *F. nucleatum*: 3.50 ± 0.58 *P. intermedia*: 3.00 ± 0 *P. micra*: 0.00 ± 0 48h *E. faecalis*: 1.94 ± 0.53 *F. nucleatum*: 3.38 ± 0.43 *P. intermedia*: 3.50 ± 0.58 *P. micra*: 4.56 ± 0.52 5 days *E. faecalis*: 2.17 ± 0.25 *F. nucleatum:* 8.50 ± 0.58 *P. intermedia*: 9.00 ± 0 *P. micra*: 4.50 ± 0.58 7 days *E. faecalis*: 2.00 ± 0 *F. nucleatum*: 8.75 ± 0.29 *P. intermedia*: 8.75 ± 0.29 *P. micra*: 4.75 ± 0.25 G2.2 24h *E. faecalis*: 1.78 ± 1.70 *F. nucleatum*: 0.00 ± 0 *P. intermedia*: 0.00 ± 0 *P. micra*: 0.00 ± 0 48h *E. faecalis*: 3.33 ± 0.25 *F. nucleatum*: 1.00 ± 0 *P. intermedia*: 0.00 ± 0 *P. micra*: 0.00 ± 0	shower the highest antibacterail activity against the examined obligate anaerobic bacterial.

					5 days *E. faecalis*: 3.83 ± 0.25 *F. nucleatum*: 1.13 ± 0.14 *P. intermedia*: 0.00 ± 0 *P. micra*: 0.00 ± 0 7 days *E. faecalis*: 3.85 ± 0.25 *F. nucleatum*: 1.25 ± 0 *P. intermedia*: 0.00 ± 0 *P. micra*: 0.00 ± 0	
Barbosa *et al*. 2020	G1 (AH Plus): 5 G2.1 (Bio-C Sealer): 5 G2.2 (EndoSequence BC Sealer): 5	NR	Biofilm of *E. faecalis* and *S. mutans*	Agar diffusion test (ADT) CFU/mL Count	Amount of viable bacteria (mean ± SD) G1 *E. faecalis*: 9.16 ± 0.72 *S. mutans*: 7.24 ± 0.73 G2.1 *E. faecalis*: 8.65 ± 0.61 *S. mutans*: 7.55 ± 0.58 G2.2 *E. faecalis*: 8.82 ± 0.56 *S. mutans*: 7.40 ± 0.74	The root canal sealer exhibit antibacterial effects similar.
Bose *et al*. 2020	G1 (AH Plus): 3 G2.1 (TotalFill BC): 3 G2.2 (BioRoot RCS): 3	NR	E. faecalis (OMGS 3202), P. acnes, A. radicidentis, S. epidermidis, S. mitis/oralis	Direct Contact Test (DCT) Planktonic suspensions Multispecies Biofilm Inhibition Test CFU/mL Count	Amount of viable bacteria (mean±SD) DCT G1 *E. faecalis* 1h: 5.98 ± 0.68 24h: 3.81 ± 0.77 168h: 3.36 ± 0.36 *A. radicidentis* 1h: 6.39 ± 0.02 24h: 3.77 ± 0.32 168h: 3.31 ± 0.71 *P. acnes* 1h: 6.63 ± 0.06 24h: 4.77 ± 0.12 168h: 4.40 ± 1.21 *S. epidermidis*	Bioceramics sealers exhibited effective antimicrobial properties. This was demonstrated by superior biofilm inhibition capacity and microbial killing, with strong alkalizing activity compared to AH Plus.

					1h: 6.23 ± 0.16 24h: 2.97 ± 0.12 168h: 2.13 ± 0.07 *S. mitis/oralis* 1h: 5.11 ± 0.57 24h: 4.39 ± 0.77 168h: 3.84 ± 0.22 G2.1 *E. faecalis* 1h: 7.71 ± 0.02 24h: 6.97 ± 0.1 168h: 3.12 ± 0.15 *A. radicidentis* 1h: 6.21 ± 0.1 24h: 4.77 ± 0.05 168h: 4.54 ± 0.12 *P. acnes* 1h: 7.75 ± 0.09 24h: 5.80 ± 0.70 168h: 4.53 ± 0.09 *S. epidermidis* 1h: 6.13 ± 0.13 24h: 5.22 ± 0.11 168h: 5.50 ± 0.10 *S. mitis/oralis* 1h: 5.77 ± 0.18 24h: 5.39 ± 0.03 168h: 4.56 ± 0.25 G2.2 *E. faecalis* 1h:7.36 ± 0.32 24h: 6.25 ± 0.09 168h: 2.74 ± 0.21 *A. radicidentis* 1h: 5.42 ± 0.38 24h: 4.21 ± 0.24 168h: 4.47 ± 0.23 *P. acnes* 1h: 5.26 ± 0.28	

					24h: 4.33 ± 0.21 168h: 2.72 ± 0.11 *S. epidermidis* 1h: 5.27 ± 0.18 24h: 3.54 ± 0.60 168h: 2.44 ± 0.23 *S. mitis/oralis* 1h: 4.73 ± 0.25 24h: 4.03 ± 0.28 168h: 3.01 ± 0.08 Multispecies Biofilm Inhibition Test G1: 10.04 ± 0.22 G2.1: 9.73 ± 0.28 G2.2: 9.08 ± 0.38	
Long *et al*. 2020	G1 (AH Plus): 12 G2 (BioRoot RCS): 12	NR	*E. faecalis* (ATCC 29212)	Live-dead staining using Confocal Laser Scanning Microscopy (CLSM)	Fraction dead (%) G1 1 week: 5% 2 weeks: 11% 4 weeks: 52% 8 weeks: 59% G2 No attached bacterial cells were identified for the BioRoot RCS	The AH Plus tested allowed biofilm formation at various stages except the BioRoot RCS, which exhibited antimicrobial properties.
Šimundić Munitić *et al*. 2020	G1 (AH Plus): 17 G2.1 (MTA Fillapex): 17 G2.2 (TotalFill BC Sealer): 17 G2.3 (BioRoot RCS): 17	NR	*E. faecalis*	CFU/mL Count	G1 60 min: 1.78 ± 6.51 G2.1 60 min: 3.51 ± 2.50 G2.2 60 min: 3.72 ± 1.20 G2.3 60 min: 3.58 ± 2.56	The Total Fill BC Sealer and AH Plus had better antibacterial efficacy than the BioRoot RCS and the MTA Fillapex sealers.

Rathod *et al*. 2020	G1 (AH Plus) G2 (EndoSequence BC Sealer)	NR	*S. aureus* (ATCC 6538) and *C. albicans* (ATCC 10231)	Agar diffusion test (ADT) Zone of inhibition (mm)	Zone of inhibition (mm) G1 / G2 (24h) S. aureus: 18.99 ± 1.11 0.97 ± 0.47 C. albicans: 21.97 ± 1.27 / 14.76 ± 1.02	AH Plus sealer exhibited a larger zone of inhibition than EndoSequence BC Sealer.
Alsubait *et al*. 2019	G1 (AH Plus): 15 G2.1 (BioRoot RCS): 15 G2.2 (TotalFill BC Sealer): 15	Semi cylindrical root dentin human single-rooted teeth	*E. faecalis* (ATCC 47077)	Bacterial viability in dentin by Confocal Laser Scanning Microscopy (CLSM) with fluorescent LIVE/DEAD BacLight Bacterial Viability stain	% of dead cells G1 1 day: 35% 7 days: 31% 30 days: 28.5% G2.1 1 day: 47,88% 7 days: 13% 30 days: 61,75% G2.2 1 day: 37% 7 days: 46,2% 30 days: 42%	Bioceramics root canal sealers possess antimicrobial effects against *Enterococcus faecalis* biofilms.
Bukhari & Karabucak 2019	G1 (AH Plus): 12 G2 (EndoSequence BC Sealer): 12	Caries-free single-rooted (uniform 4-mm dentin cylinder)	*E. faecalis* (OG1RF)	Confocal Laser Scanning Microscopic (CLSM)	% of Dead Cell G1 / G2 24 hours: 26.07 ± 5.956 / 76.33 ± 5.348 2 weeks: 25.84 ± 6.906 / 74.79 ± 6.612	EndoSequence BC Sealer exhibited significant antimicrobial capacity in the presence of dentin for up to 2 weeks on an 8-week-old *E. faecalis* biofilm in comparison with AH Plus sealer.
Pawińska *et al*. 2019	G1 (AH Plus): 3 G2 (MTA Fillapex): 3	NR	*Actinomyces israelii* (NCTC 8047) and *Actinomyces viscosus* (ATCC 15987)	Agar diffusion test (ADT) Zones of bacterial growth inhibition (mm)	G1 / G2 *Actinomyces viscosus* 96 hours: 15.00 ± 1 / 32.33 ± 0.58 1 week: 14.67 ± 0.58 / 30.67 ± 0.58 2 weeks: 15.00 ± 0.00 / 28.00 ± 0.00 3 weeks: 15.00 ± 0.00 / 28.00 ± 0.00 4 weeks: 15.00 ± 0.00 / 28.33 ± 0.58 *Actinomyces israelii* 96 hours: 12.67 ± 0.58 / 29.67 ± 0.58 1 week: 13.67 ± 0.58 28.33 ± 0.58 2 weeks: 15.00 ± 0.00 / 29.00 ± 0.00 3 weeks: 16.00 ± 0.00 / 30.67 ± 0.58 4 weeks: 16.33 ± 0.58 / 31.67 ± 0.58	The MTA Fillapex showing the highest antibacterial effect on both bacterial strains.

Zordan-Bronzel *et al*. 2019	G1 (AH Plus): 8 G2.1 (TotalFill BC Sealer): 8 G2.2 (Bioceramic Experimental): 8	NR	*E. faecalis* (ATCC 29212) *C. albicans* (ATCC 10231)	CFU/mL Count	Amount of viable bacteria (mean ± SD) G1 *E. faecalis*: 7.283 ± 0.229 *C. albicans*: 2.784 ± 0.443 *E. faecalis* biofilm: 7.426 ± 0.100 G2.1 *E. faecalis*: 4.226 ± 0.808 *C. albicans*: 0.0 ± 0.0 *E. faecalis* biofilm: 4.966 ± 0.530 G2.2 *E. faecalis*: 4.881 ± 0.668 *C.albicans*: 0.0 ± 0.0 *E. faecalis* biofilm: 4.914 ± 0.583	The bioceramics showed better effects for antimicrobial activity.
Dalmia *et al*. 2018	G1 (AH Plus) G2 (MTA Fillapex)	NR	*E. faecalis* (MTCC 2093	Diameter of inhibition zone (mm)	G1 / G2 24 hours: 9 / 6.66 48 hours: 7.66 / 4.3 72 hours: 7.33 / 0	MTA Fillapex showed the least antimicrobial activity.
Kapralos *et al*. 2018	G1 (AH Plus) G2 (TotalFill BC Sealer)	NR	*E. faecalis* (ATCC) 19434, *S. mutans* ATCC 700610, *S. epidermidis* ATCC 35984, and *S. aureus* Newman	CFU/mL Count	Amount of viable bacteria G1 / G2 *E. faecalis* Freshly mixed: 0 / 0.589 24 hours: 5.668 / 0 7 days: 6.076 / *S. mutans* Freshly mixed: 0 / 0.54 24 hours: 5.182 / 0 7 days: 4.94 / 0 *S. epidermidis* Freshly mixed: 0 / 0 24 hours: 5.734 / 0 7 days: 6.072 / *S. aureus* Newman Freshly mixed: 0 / 0 24 hours: 6.456 / 0 7 days: 6.554 / 0 Biofilm assays (1 hour)	Bacteria in biofilms showed higher susceptibility for AH Plus compared with TotalFill BC sealer during the first 24 hours after setting.

					G1 / G2 *E. faecalis*: 0 / 3.785 *S. mutans*: 1.355 / 1.456 *S. epidermidis*: 0 / 1.352 *S. aureus* Newman: 1.868 / 4.961	
Arias-Moliz & Camilleri 2016	G1 (AH Plus): 5 G2.1 (BioRoot RCS): 5 G2.2 (MTA Fillapex): 5	NR	*E. faecalis* (ATCC 29212)	Agar diffusion test (ADT) and Confocal laser scanning microscopy (CLSM)	ADT G1 Water: 0 EDTA: 17.5 ± 1.5 PBS: 0 G2.1 Water: 0 EDTA: 17.2 ± 1.1 PBS: 0 G2.2 Water: 0 EDTA: 8 ± 0.9 PBS: 0 Percentage of dead cells Confocal laser scanning microscopy (CLSM) G1 Water: 29.98 ± 26.72 EDTA: 41.37 ± 14.91 PBS: 25.16 ± 22.09 G2.1 Water: 71.67 ± 15.03 EDTA: 78.97 ± 27.74 PBS: 58.39 ± 17.05 G2.2 Water: 52.90 ± 25.68 EDTA: 47.99 ± 16.49 PBS: 27.79 ± 18.76	The root canal sealers exerted a higher antimicrobial activity when EDTA was used as final irrigant. BioRoot RCS showed the greatest antimicrobial activity followed by MTA Fillapex against bacteria-infected dentine.
Candeiro *et al*. 2016	G1 (AH Plus): 10		*E. faecalis*	Agar diffusion test (ADT) and Direct contact test (DCT)	DCT (Mean CFU mL^{-1}) G1 / G2 1 hour: 0.0 / 4.5 ± 04 24 hours: 0.0 / 0.0 72 hours: 0.0 / 0.0	

	G2 (Endosequence BC sealer): 10	NR	(ATCC 29212)	CFU/mL Count	168 hours: 0.0 / 0.0 ADT (mm) G1 / G2 10.31 ± 0.21 / 6.00 ± 0.03	The both root canal sealers a similar antibacterial effect against *E. faecalis*.
Shakya *et al*. 2016	G1 (AH Plus): 10 G2 (MTA Fillapex): 10	NR	*E. faecalis* (ATCC 29212)	Agar diffusion test (ADT) and Direct Contact Test (DCT)	ADT (mm inibition) G1 / G2 24 hours: 4.15 ± .02 / 13.5 ± .01 7 days: 2.15±0.23 / 11.12 ±.25 DCT (no of organisms/ml) G1 / G2 24 hours: 5.7 $\times 10^2$ ± .02 / 5.02 $\times 10^2$ ± .01 7 days: 3.64 $\times 10^2$ ± .013 / 2.73 $\times 10^2$ ± .017	MTA Fillapex showed the greatest antimicrobial activity.
Du *et al*. 2016	G1 (AH Plus): 6 G2.1 (Endosequence BC Sealer): 6 G2.2 (MTA Fillapex): 6	Single-rooted caries-free teeth	*E. faecalis* (VP3-181)	Confocal Laser Scanning Microscopic (CLSM)	Proportion of Dead Cell G1 5% NaOCl 7 days: 0.68 ± 0.04 30 days: 0.74 ± 0.03 60 days: 0.77 ± 0.02 Sterile Water 7 days: 0.30 ± 0.02 30 days: 0.39 ± 0.02 60 days: 0.41 ± 0.01 G2.1 5% NaOCl 7 days: 0.67 ± 0.05 30 days: 0.75 ± 0.05 60 days: 0.76 ± 0.04 Sterile Water 7 days: 0.27 ± 0.03 30 days: 0.40 ± 0.03 60 days: 0.40 ± 0.04 G2.2 5% NaOCl 7 days: 0.73 ± 0.03 30 days: 0.81 ± 0.03	The results of the present study indicate that the sequential use of NaOCl and root canal sealers kills more bacteria in infected dentin than either one of them used alone. MTA Fillapex exhibited the highest effectiveness against *E. faecalis* both when used alone or after NaOCl although the difference to BC Sealer or AH Plus was small.

					60 days: 0.83 ± 0.02 Sterile Water 7 days: 0.41 ± 0.02 30 days: 0.53 ± 0.04 60 days: 0.55 ± 0.04	
Weckwerth *et al*. 2015	G1 (AH Plus) G2 (MTA Fillapex)	NR	*C. albicans* (ATCC 10231)	Agar diffusion test (ADT)	Inhibition halos (mm) G1 24 hours: 8.1±1.26 / 11.1±1.55 48 hours: 8.2 ± 1.10 / 11.1 ± 1.26	Both root canal sealers have similar antifungal activity.
Nirupama *et al*. 2014	G1 (AH Plus) G2 (iRoot SP)	NR	*E. faecalis* (ATCC 29212), *C. albicans* (ATCC 90028), and *S. aureus* (ATCC 25923)	Direct Contact Test (DCT) CFU/mL Count	-------	AH Plus to have highest antimicrobial activity against both bacteria (*E. faecalis and S. aureus*) and yeast (*C. albicans*).
Wang *et al*. 2014	G1 (AH Plus): 6 G2 (Endosequence BC Sealer): 6	Single-rooted teeth	*E. faecalis* (VP3-181)	Confocal Laser Scanning Microscopic (CLSM)	Proportion of Dead Cell G1 / G2 1 day: 0.21 ± 0.10 / 0.18 ± 0.09 7 days: 0.33 ± 0.12 / 0.35 ± 0.11 30 days: 0.46 ± 0.14 / 0.45 ± 0.13	The both endodontic root canal sealers had antibacterial effects against *E. faecalis* in the dentinal tubules.
Faria-Júnior *et al*. 2013	G1 (AH Plus): 8 G2.1 (MTA Fillapex): 8 G2.2 (Bioceramic Experimental): 8	Bovine central incisors	*E. faecalis* (ATCC 29212)	direct contact test (DCT) CFU/mL Count	2 days post-manipulation G1 5 hours: 7.8 10 hours: 6.9882 15 hours:8.1680 G2.1 5 hours: 6.5161 10 hours: 6.3539 15 hours: 4.7971 G2.2 5 hours: 7.4927 10 hours: 7.4374 15 hours: 6.2381 7 days post-manipulation G1 5 hours: 7.2842	MTA Fillapex were able to significantly reduce the number of bacteria in biofilms.

					10 hours: 7.2981 15 hours: 7.1329 G2.1 5 hours: 5.6121 10 hours: 5.3445 15 hours: 4.6487 G2.2 5 hours: 7.2196 10 hours: 6.9401 15 hours: 6.9423	
Ozcan *et al*. 2013	G1 (AH Plus) G2.1 (iRoot SP) G2.2 (MTA Fillapex)	NR	*C. albicans* (ATCC 10231)	Direct contact test (DCT) CFU/mL Count	Amount of viable cell G1 20 min: 0 1 day: 4.94 7 days: 5.18 G2.1 20 min: 3.78 1 day: 4.68 7 days: 5.02 G2.2 20 min: 5.74 1 day: 5.68 7 days: 5.6	All sealers exhibited antifungal activity when freshly mixed, but AH Plus had very potent antifungal activity.
Willershausen *et al*. 2011	G1 (AH Plus) G2 (EndoSequence BC Sealer)	NR	*E. faecalis* (DSM 20478) and *Parvimonas micra* (ATCC 33270)	scanning electron microscopy (SEM)	------------	Endosequence BC had no antibacterial effect against *E. faecalis*.

NR = not reported.

Table 4. Assessment of the risk of bias of the included studies

Dentinal tubule penetration studies	***Clearly stated objective***	***Sample size justification***	***Randomization of samples***	***Possibility of comparison between control and experimental groups***	***Statistical analysis carried out***
Alim Uysal *et al*. 2021	Low	High	Low	Low	Low
Caceres *et al*. 2021	Low	High	Low	Low	Low
Eskander *et al*. 2021	Low	High	Low	Low	Low
Furtado *et al*. 2021	Low	Low	Low	Low	Low
Kakani *et al*. 2021	Low	High	Unclear	Low	Low
Khalil & Mahdee 2021	Low	High	Unclear	Low	Low
Muedra *et al*. 2021	Low	High	Low	Low	Low
Yang *et al*. 2021	Low	Low	Low	Low	Low
Coronas *et al*. 2020	Low	High	Low	Low	Low
De Bem *et al*. 2020	Low	High	Low	Low	Low
Giovarruscio *et al*. 2020	Low	High	Unclear	Low	Low
Turkyilmaz & Erdemir 2020	Low	High	Low	Low	Low
Candeiro *et al*. 2019	Low	High	Unclear	Low	Low
El Hachem *et al*. 2019	Low	High	Low	Low	Low
Gunes *et al*. 2019	Low	High	Unclear	Low	Low
Kim *et al*. 2019	Low	High	Unclear	Low	Low
Jardim Del Monaco *et al*. 2018	Low	High	Low	Low	Low
Piazza *et al*. 2018	Low	High	Low	Low	Low
Chen *et al*. 2017	Low	High	Low	Low	Low
Cruz *et al*. 2017	Low	High	Low	Low	Low
Menezes *et al*. 2017	Low	High	Unclear	Low	Low
Akcay *et al*. 2016	Low	High	Low	Low	Low
Sonu *et al*. 2016	Low	High	Low	Low	low
Nikhil *et al*. 2015	Low	High	Low	Low	Low

Silva *et al*. 2015	Low	High	Unclear	Low	Low
Amoroso-Silva *et al*. 2014	Low	High	Low	Low	Low
Kara Tuncer *et al*. 2014	Low	High	Low	Low	Low
Antimicrobial activity studies	**Clearly stated objective**	**Sample size justification**	**Randomization of samples**	**Possibility of comparison between control and experimental groups**	**Statistical analysis carried out**
Caldas *et al*. 2021	Low	Low	Low	Low	Low
Carvalho *et al*. 2021	Low	Low	Low	Low	Low
Kapralos *et al*. 2021	Low	Low	Low	Low	Low
Marashdeh *et al*. 2021	Low	Low	Low	Low	Low
Zancan *et al*. 2021	Low	Low	Low	Low	Low
Allahem *et al*. 2020	Low	Low	Low	Low	Low
Barbosa *et al*. 2020	Low	Low	Low	Low	Low
Bose *et al*. 2020	Low	Low	Low	Low	Low
Long *et al*. 2020	Low	Low	Low	Low	Low
Šimundić Munitić *et al*. 2020	Low	Low	Low	Low	Low
Rathod *et al*. 2020	Low	Low	Low	Low	Low
Alsubait *et al*. 2019	Low	Low	Low	Low	Low
Bukhari & Karabucak 2019	Low	Low	Low	Low	Low
Pawińska *et al*. 2019	Low	Low	Low	Low	Low
Zordan-Bronzel *et al*. 2019	Low	Low	Low	Low	Low
Dalmia *et al*. 2018	Low	Low	Low	Low	Low
Kapralos *et al*. 2018	Low	Low	Low	Low	Low
Arias-Moliz & Camilleri 2016	Low	Low	Low	Low	Low
Candeiro *et al*. 2016	Low	Low	Low	Low	Low
Shakya *et al*. 2016	Low	Low	Low	Low	Low
Du *et al*. 2016	Low	Low	Low	Low	Low
Weckwerth *et al*. 2015	Low	Low	Low	Low	Low
Nirupama *et al*. 2014	Low	Low	Low	Low	Low
Wang *et al*. 2014	Low	Low	Low	Low	Low

Faria-Júnior *et al*. 2013	Low	Low	Low	Low	Low
Ozcan *et al*. 2013	Low	Low	Low	Low	Low
Willershausen *et al*. 2011	Low	Low	Low	Low	Low

Figure 1. PRISMA Flowchart – Flow diagram showing the entre search process

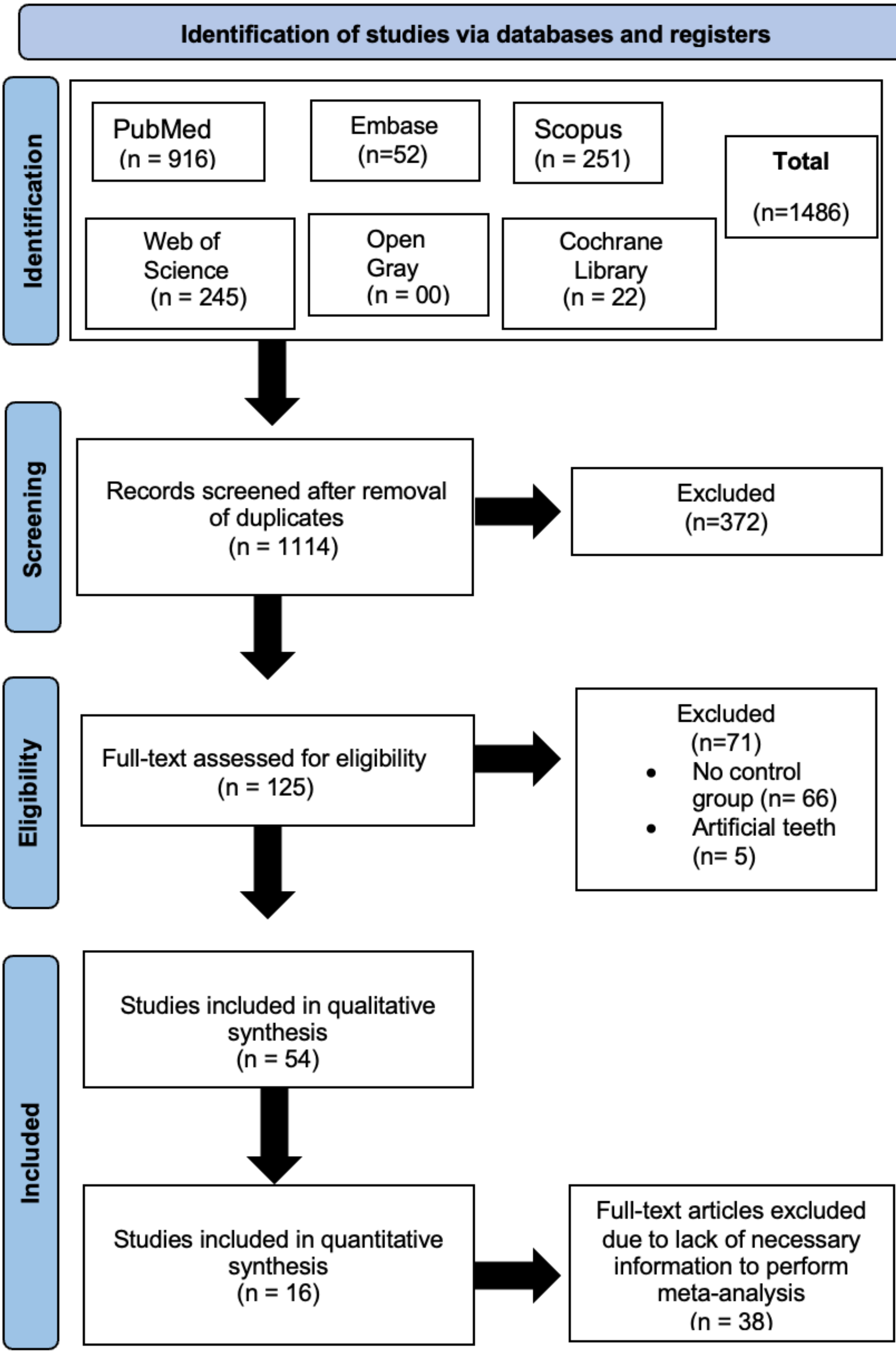


Figure 2. Meta-analysis of bioceramics x AH Plus®. Coronal - A: Meta-analysis; B: publication bias analysis (trim-and-fill); C: funnel plot. Middle - D: Meta-analysis; E: publication bias analysis (trim-and-fill); F: funnel plot. Apical - G: Meta-analysis; H: publication bias analysis (trim-and-fill); I: funnel plot

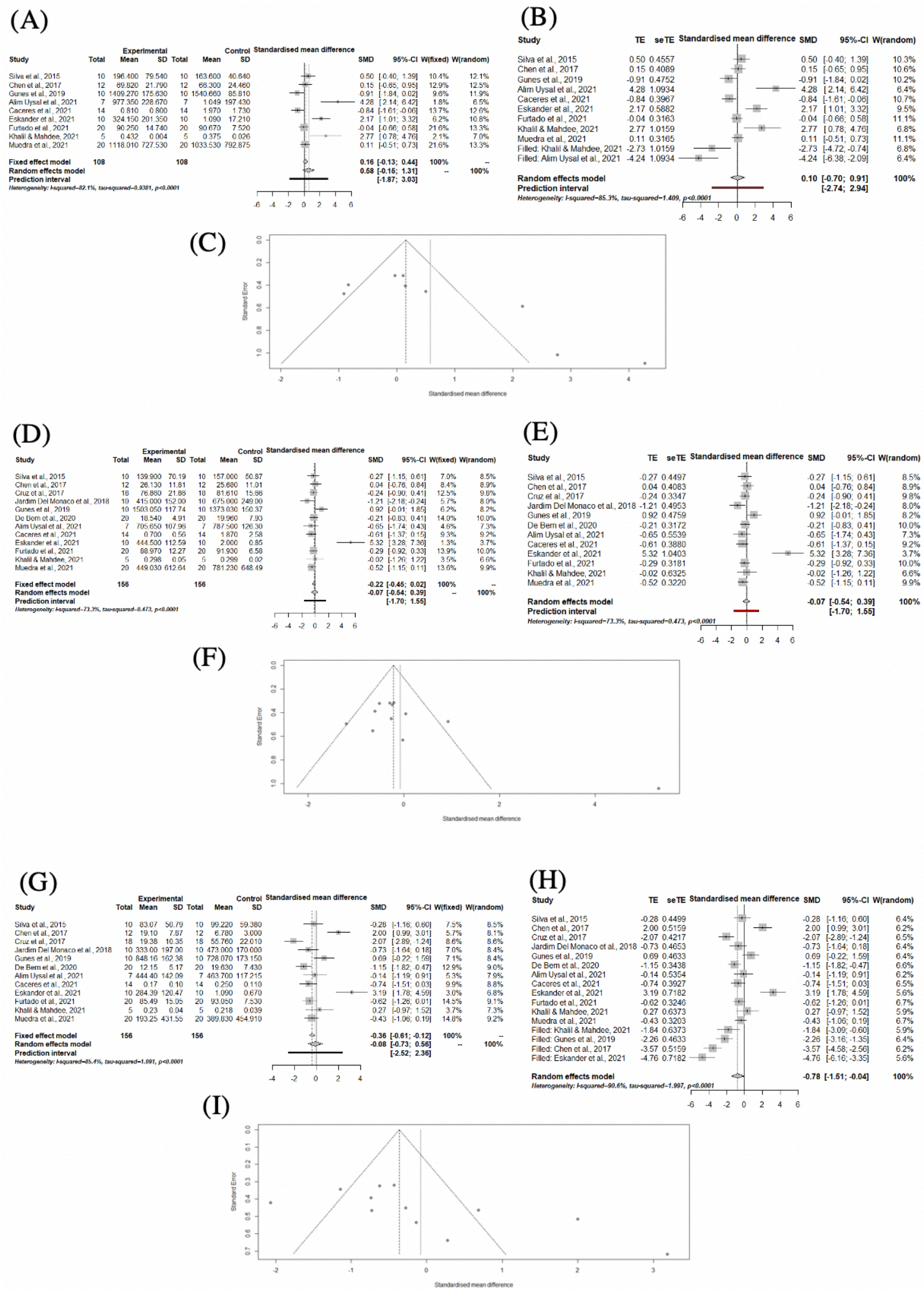


Figure 3. Meta-analysis of MTA Fillapex x AH Plus®. Coronal - A: Meta-analysis; B: publication bias analysis (trim-and-fill). Middle - C: Meta-analysis; D: publication bias analysis (trim-and-fill). Apical - E: Meta-analysis; F: publication bias analysis (trim-and-fill).

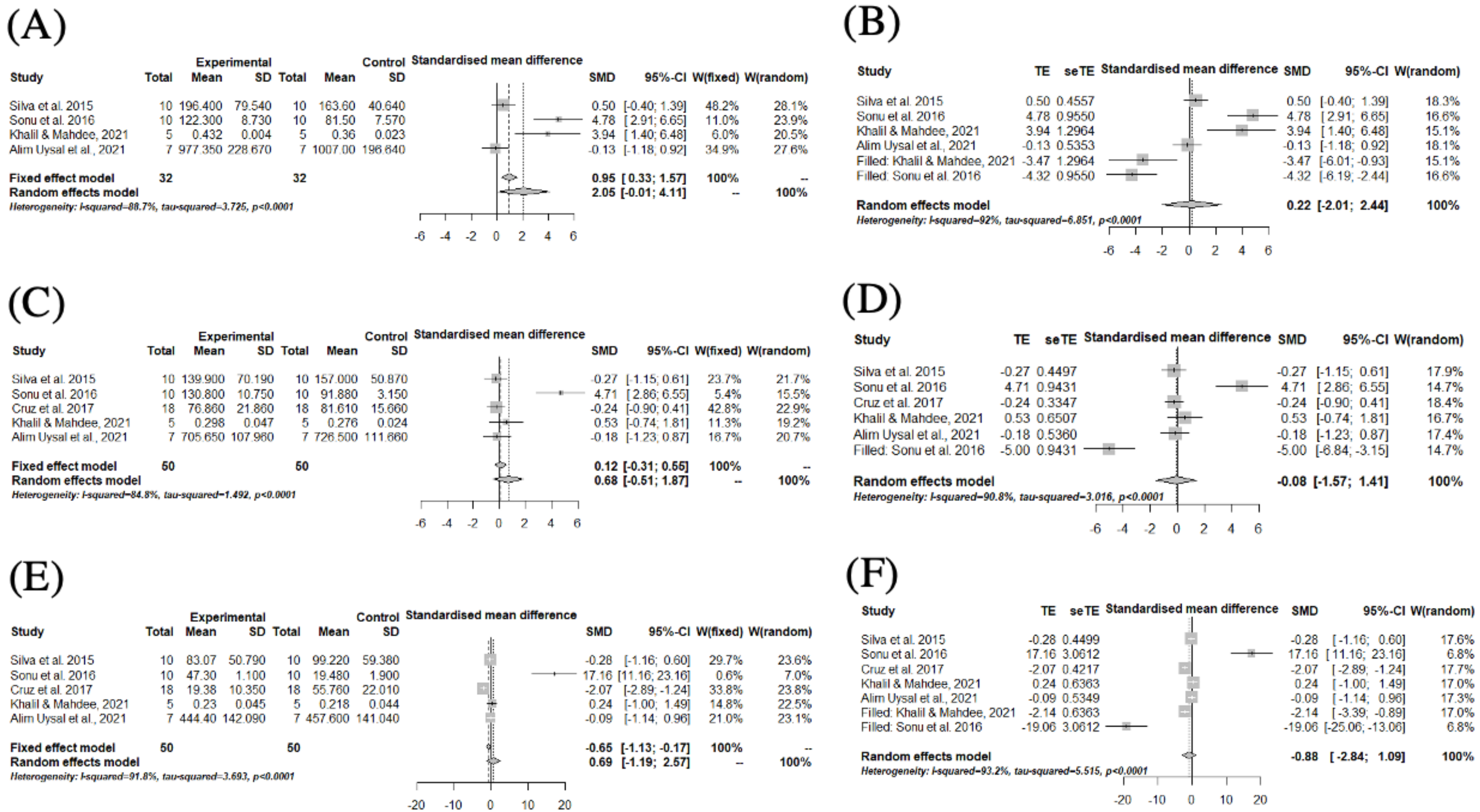


Figure 4. Meta-analysis of EndoSequence BC Sealer x AH Plus®. Coronal - A: Meta-analysis; B: publication bias analysis (trim-and-fill). Middle - C: Meta-analysis; D: publication bias analysis (trim-and-fill). Apical - E: Meta-analysis; F: publication bias analysis (trim-and-fill).

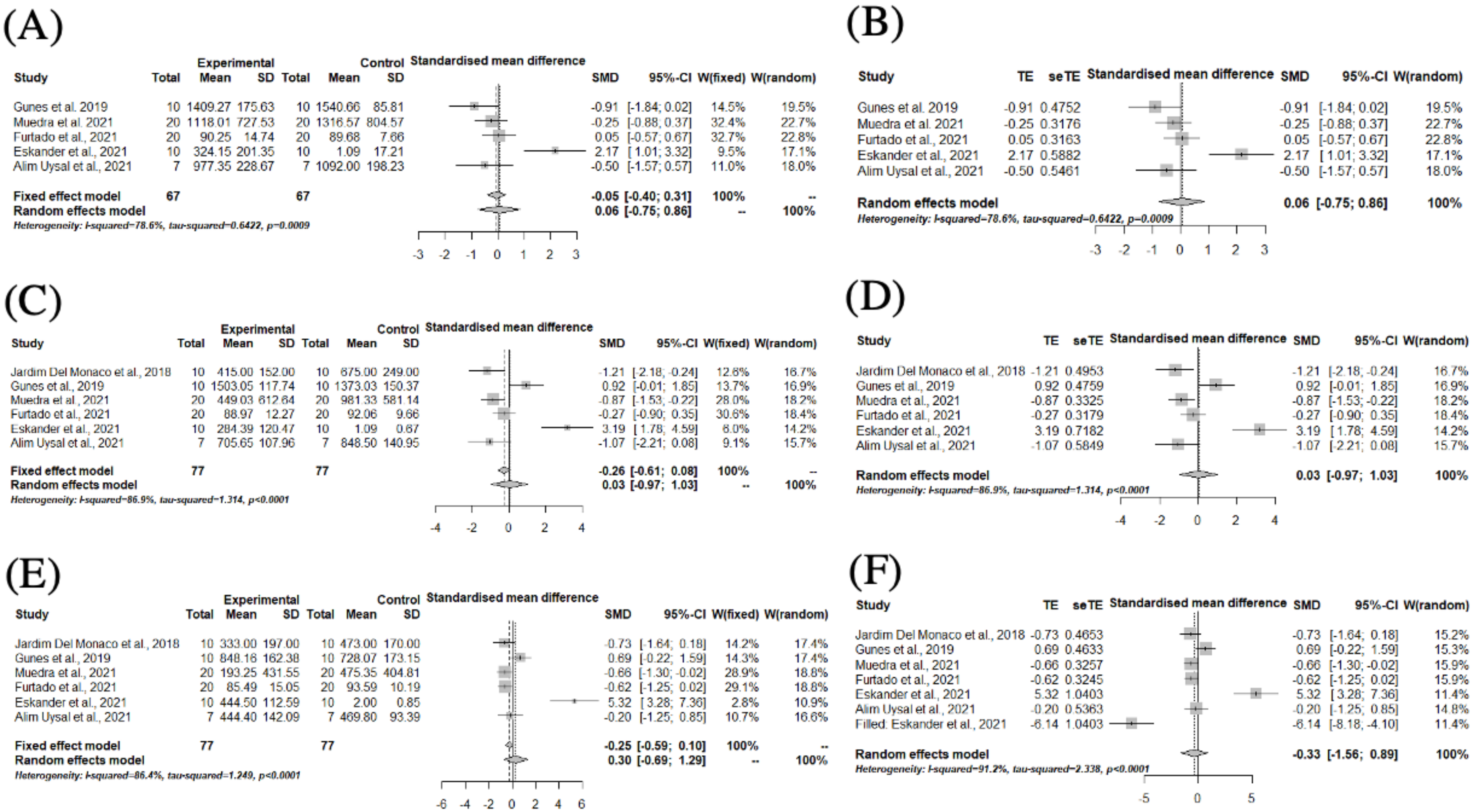
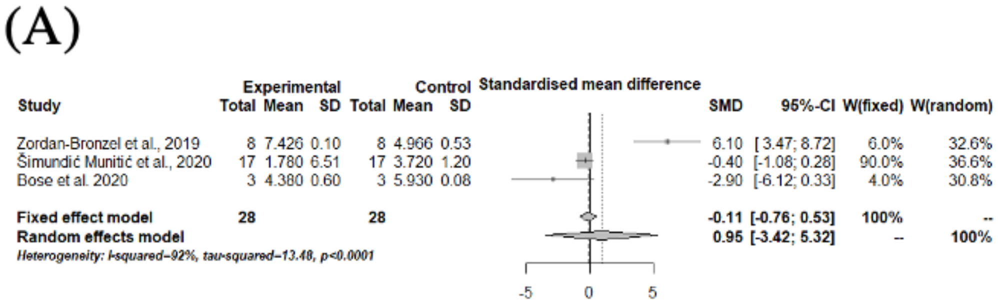
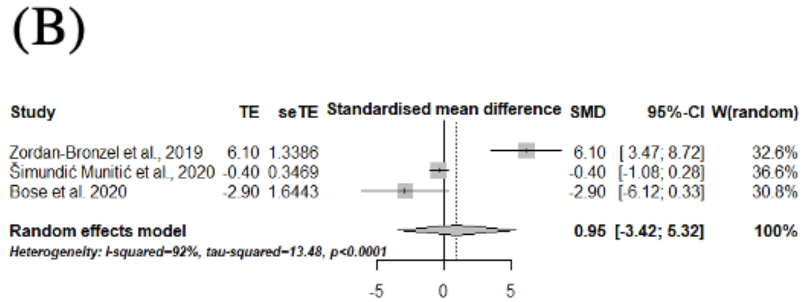
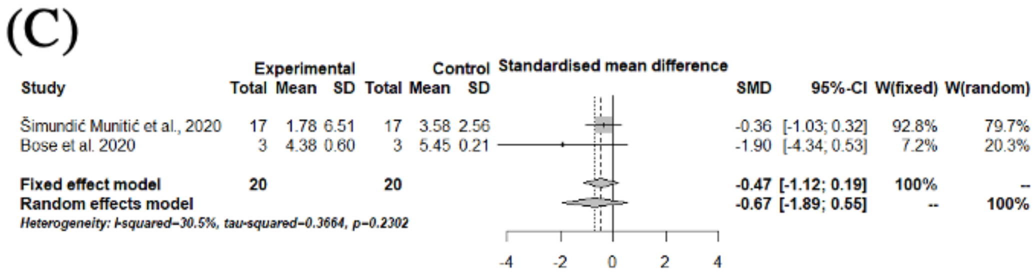


Figure 5. Meta-analysis antimicrobial activity. A: Meta-analysis of TotalFill BC Sealer x AH Plus®; B: publication bias analysis (trim-and-fill) of TotalFill BC Sealer x AH Plus®. C: Meta-analysis of BioRoot RCS x AH Plus®.







Conclusão Geral

4 CONCLUSÃO GERAL

Dentro das limitações desses trabalhos de revisões sistemáticas e meta-análises, pode-se concluir que os cimentos biocerâmicos apresentam menor dor pós operatória nas primeiras 24 e 48 horas, e apresentam respostas similares para penetração em túbulos dentinários e atividade antimicrobiana quando comparado ao cimento AH Plus®.

Referências Introdução Geral

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