

Antimicrobial Activity of Bioceramic Root Canal Sealers: A Systematic Review

Actividad Antimicrobiana de los Cementos Endodónticos Biocerámicos:
una Revisión Sistemática

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ABSTRACT: Actually, bioceramic endodontic sealers represent an advantageous option for root canals sealing due to their multiple properties. There are several in vitro studies about their antimicrobial properties, however, their comparative antimicrobial potential, compared with other types of endodontic sealers is not entirely clear. The aim of the present research was to perform a systematic review to assess the antimicrobial effectiveness of commercially available bioceramic root canal sealers against *Enterococcus faecalis* in accordance with the PRISMA statement. A literature search was made in four electronic databases: MEDLINE, ClinicalKey, ScienceDirect and EBSCO. Were included in vitro studies, published in English, between 2015 and 2019. The evaluation of the selected studies was performed by two authors, independently. Four studies were included in the review. EndoSequence™ (Brasseler USA, Savannah, GA) also available on the market as TotalFill™ (Brasseler USA, Savannah, GA) and BioRoot™ were the bioceramic root canal sealers evaluated. The risk of bias was assessed according to the modified CONSORT checklist for preclinical studies. Selected studies reported positive antimicrobial activity against *Enterococcus faecalis* in the bioceramic sealers evaluated. The less activity was observed on BioRoot™ sealer. All studies used a planktonic form of *Enterococcus faecalis* and in vitro laboratory methods of evaluation. The antimicrobial activity reported varies according to the laboratory method used, even for the same sealer, so the existence of false negatives is suggested. This systematic review identified the need for more research into the antimicrobial activity of bioceramic sealers, ideally with models using bacterial biofilms and studies in humans.

KEY WORDS: anti-infective agent, bacteria, biofilms, endodontics, enterococcus faecalis, root canal sealer.

INTRODUCTION

The success of endodontic treatment depends on proper instrumentation and irrigation of root canals, with the objective of to eliminate microorganisms that may be found in them, followed by a correct root canal filling (Nirupama *et al.*, 2014). Although instrumentation and irrigation decrease the number of microorganisms, it is impossible to eliminate all of them from the root canal system in clinical conditions (Zhang *et al.*, 2009; Oporto *et al.*, 2018). Persisting biofilms in the endodontically treated root canal may lead to periapical or periodontal disease (Wang *et al.*, 2014a). Thus, one of the biggest challenges in endodontic treatments is

to eliminate or reducing the bacterial biofilms of the root canal system to levels that are compatible with dental and systemic health. Root filling is therefore a critical step of root canal treatment (Nirupama *et al.*), preferably using dental materials with antimicrobial properties (Wang *et al.*, 2014b). The use of root canal sealers with antimicrobial activity is considered beneficial in reducing the concentration of residual microorganisms (Baer & Maki, 2010; Nirupama *et al.*; Kapralos *et al.*, 2018), preventing recurrent root canal infection and assisting the healing of periapical tissues (Singh *et al.*, 2016). Previous studies have investigated

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the antimicrobial activity of root canal sealers (Alshwaimi, 2011) and reported that their freshly mixed are effective against some microorganism, however their antimicrobial effectiveness between 2 and 7 days later has not been reported (Morgental *et al.*, 2011). Within this research, an important number has focused on the comparison of the various materials *in vitro* (Wang *et al.*, 2014b). Root canal sealers have been reviewed based on their composition, including sealers based on zinc oxide eugenol, calcium hydroxide, glass ionomer, and resin. However, no extensive review of bioceramic-based sealers has been conducted (Al-Haddad & Che Ab Aziz, 2016).

Bioceramic-based root canal sealers have been available for use in endodontics for the past thirty years; the first documented use of a bioceramic as a root canal sealer was an experimental calcium phosphate sealer used in 1984 (Krell & Wefel, 1984). Today, bioceramics-based root canal sealers have demonstrated an advantage when comparing with other endodontic sealers: their biocompatibility, evidenced in the decreased capacity of these sealers to induce adverse reaction in peripheral tissues (i.e. toxicity, irritation, inflammation, allergy, or carcinogenicity) (Al-Haddad & Che Ab Aziz) in episodes of extrusion sealer in periapical zone. This property is based on their calcium phosphate content, which results in a chemical composition and crystalline structure similar to tooth and bone apatite materials (Koch & Brave, 2009).

The available studies have demonstrated that *Enterococcus faecalis* is one of the microorganisms present in devitalized teeth, especially in teeth with recurrent endodontic infection (Oporto *et al.*). It is the most common intra-radicular microorganism isolated from periapical periodontitis (Rodríguez-Niklitschek & Oporto, 2015; Oporto *et al.*) and is therefore commonly used to test the antibacterial activity of root canal sealers. Other microorganisms, such as *Micrococcus luteus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Streptococcus mutans*, have also been used to test the antibacterial effects of endodontic sealers (Al-Haddad & Che Ab Aziz), but *Enterococcus faecalis* is relevant because several studies report prevalence of up to 77 % in teeth with failed endodontic treatment (Singh *et al.*). Thus analyzing the antibacterial action of root canal sealers against this bacterium is relevant to clinical practice. Three methods are commonly used to evaluate the antibacterial activity of root canal sealers: agar diffusion test (ADT), direct contact test

(DCT) and modified direct contact test (MDCT) (Poggio *et al.*, 2017; Kapralos *et al.*).

The aim of this systematic review was to provide knowledge synthesis of the available evidence regarding about antimicrobial properties of bioceramic endodontic root canal sealers in presence of *Enterococcus faecalis*, specifically evaluated with *in vitro* studies.

MATERIAL AND METHOD

Search strategy. An electronic systematic search was performed for available evidence reporting the antimicrobial activity of bioceramic root canal sealers, following the PRISMA checklist (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) (Moher *et al.*, 2009) (Fig. 1). The search was performed using four electronic databases: MEDLINE, ClinicalKey, ScienceDirect and EBSCO (Dentistry and Oral Sciences sources databases). Search was made using a limit of time, between January 2015 and December 2019. The search strategy was made by combining specific search terms related to bioceramic endodontic sealers (examples of the search strategy used are in Table I). Only articles in English were considered. Search results were exported into a Microsoft Excel® table and they were subsequently evaluated.

Study selection. Two reviewers (C.R.N and G.H.O) independently assessed titles and abstracts obtained by the electronic search to select the relevant articles. After the removal of duplicate publications, title reviews, and abstract selection, 13 full-text articles were screened. Full texts of these studies were obtained and analyzed for eligibility by two authors independently (C.R.N and G.H.O). The following inclusion criteria were considered: studies that evaluated the antimicrobial activity of bioceramic root canal sealers, compared with other commonly used endodontic sealers; *Enterococcus faecalis* was the bacteria used in the antimicrobial evaluation and the antimicrobial activity was evaluated by agar diffusion test (ADT), direct contact test (DCT) or modified direct contact test (MDCT) *in vitro* test. Only *in vitro* studies were considered; no studies in humans were found. Studies that evaluated antimicrobial activity using planktonic cells of *Enterococcus faecalis* were selected, to avoid possible errors due to misconducted microorganisms detection in bacteria isolated from

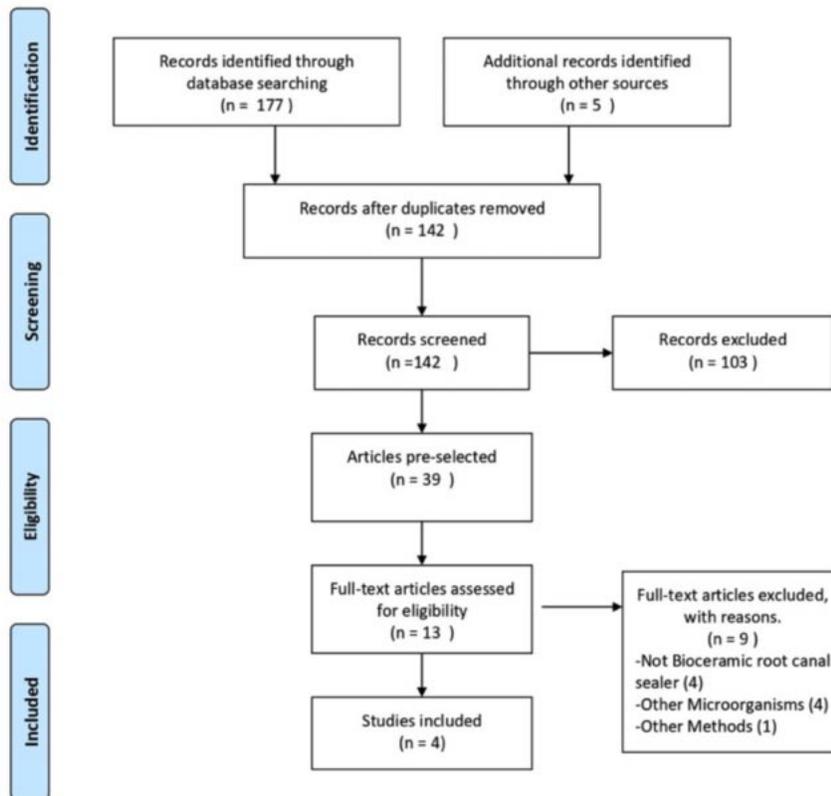


Fig. 1. PRISMA Flow diagram of the systematic review process.

Table I. Example of strategy search combinations used in PubMed.

Number of strategy	Words and terms of search	Number of results
1	bioceramic root canal sealer OR bioceramic root canal sealers OR bioceramic endodontic sealer OR bioceramic endodontic sealers	49
2	antimicrobial activity OR antimicrobial effect OR antimicrobial effectiveness OR antibacterial activity OR antibacterial effect OR antibacterial effectiveness OR anti-biofilm activity OR anti-biofilm effectiveness	25767
3	bioceramic root canal sealer OR bioceramic root canal sealers OR bioceramic endodontic sealer OR bioceramic endodontic sealers AND (antimicrobial activity OR antimicrobial effect OR antimicrobial effectiveness OR antibacterial activity OR antibacterial effect OR antibacterial effectiveness OR anti-biofilm activity OR anti-biofilm effectiveness)	5

root canals. Any possible discrepancies in the screening process were resolved via discussion or by involving a third reviewer (P.C). Finally, 4 full-text articles were selected and reviewed by the authors. The reviewers independently identified and categorized the available information in the studies.

Assessment of risk of bias. The methodological quality of the selected articles was evaluated based on the modified Consolidated Standards of Reporting

Trials (CONSORT) for in vitro preclinical studies (Faggion Jr., 2012). Eight parameters were included: intervention, outcomes, sample size, randomization, implementation, blinding, statistical methods and results. Two independent reviewers (C.R.N and G.H.O) analyzed each article. The items received a “Yes” when the authors explicitly described the corresponding parameter; if any information was not available or fully declared the item received a “No”. A third reviewer (P.C) resolved discrepancies during the review process.

RESULTS

Search and selection. From the electronic search, 182 abstracts were obtained; 39 duplicates and 103 articles not relevant to the topic were eliminated. 39 studies were pre-selected and full texts of 13 studies were evaluated. 9 studies were excluded for non-compliance with the inclusion criteria. Finally, 4 articles fulfilled the inclusion criteria (Candeiro *et al.*, 2016; Singh *et al.*; Colombo *et al.*, 2018; Kapralos *et al.*). Selected studies used the materials according to the manufacturer's instructions and recommendations. Authors evaluated growth of *Enterococcus faecalis* (ATCC 29212 and ATCC 19434) using ADT (Candeiro *et al.*; Singh *et al.*; Colombo *et al.*), DCT (Candeiro *et al.*; Colombo *et al.*) and MDCT (Kapralos *et al.*). The laboratory stages were detailed described in all the studies.

Risk of bias. As mentioned, the methodological quality of the articles included in this research was assessed by checking the CONSORT criteria (Table II). Parameters related to the intervention were described successfully in each article as well as the statistical methods used (100 %); outcomes were considered in 3 articles (75 %). Items associated with sample size, randomization and blinding were not correctly reported or were not included in the selected articles.

Root canal sealers evaluated. The study performed by Candeiro *et al.* compared the antibacterial activity between two root canal sealers: EndoSequence™ sealer (Brasseler, Savannah, GA, USA) and AH Plus™ sealer (Dentsply International Inc, York, PA, USA). EndoSequence™ is a premixed bioceramic-based root

canal sealer (Camilleri, 2016; Kakoura & Pantelidou, 2018). AH Plus™ sealer is a paste-paste system, epoxy resin-based root canal sealer (Candeiro *et al.*). Sight *et al.*, compared the antibacterial activity of different endodontic sealers. Among them, the bioceramic sealer investigated was also EndoSequence™ and were considered four root canal sealers with other composition (Singh *et al.*). Colombo *et al.* analyzed the biological and the physico-chemical properties of six endodontic sealers. Two bioceramic-based root canal sealers were evaluated: BioRoot™ (Septodont, Saint-Maur-des-Fosses, France) and TotalFill™ (Colombo *et al.*). Kapralos *et al.* evaluated the antibacterial effect of four endodontic sealers against bacteria planktonic grown. The bioceramic root sealer included was TotalFill™. Roeko™ Seal served as a positive control in this study, because the manufacturer states that it does not possess antibacterial activity (Kapralos *et al.*). The detail of all root canal sealers evaluated in the four selected studies is showed in Table III.

Antimicrobial activity of endodontic sealers. EndoSequence™ bioceramic root canal sealer is a premixed injectable bioceramic paste, with hydrophilic properties. Other brands of this type of sealer are TotalFill™ and iRoot SP™ (Camilleri; Kakoura & Pantelidou). Their composition is based on calcium silicate (Debelian & Trope, 2016; Jafari & Jafari, 2017). According to the results, the antibacterial activity of this sealer remains inconclusive. Candeiro *et al.* analyzed their antibacterial activity on *Enterococcus faecalis*

Table II. Evaluation of the studies using the modified CONSORT checklist (Figgion, 2012).

Author	1	2	3	4	5	6	7	8
Singh <i>et al.</i> (2016)	YES	NO	NO	NO	NO	NO	YES	NO
Candeiro <i>et al.</i> (2016)	YES	YES	NO	NO	NO	NO	YES	NO
Colombo <i>et al.</i> (2018)	YES	YES	NO	NO	NO	NO	YES	NO
Kapralos <i>et al.</i> (2018)	YES	YES	NO	NO	NO	NO	YES	NO

1) The intervention for each group, including how and when it was administered, with sufficient detail to enable replication.

2) Completely defined, pre-specified primary and secondary measures of outcome, including how and when they were assessed.

3) How sample size was determined.

4) Method and Mechanism used to generate the random allocation sequence.

5) Who generated the random allocation sequence, who enrolled teeth?

6) If done, who was blinded after assignment to intervention (for example, care providers, those assessing outcomes), and how and who assigned teeth to intervention.

7) Statistical methods used to compare groups for primary and secondary outcomes.

8) For each primary and secondary outcome, results for each group, and the estimated size of the effect and its precision.

Table III. Root canal sealers analyzed in the selected studies.

Autor	Bioceramic root canal sealer	Comparison	
		Root canal sealer	Type
Candeiro <i>et al.</i> , 2016	-EndoSequence BC Sealer (Brasseler, Savannah, GA, USA)	-AH Plus (Dentsply International Inc, York, PA USA)	Epoxy resin-based
Singh <i>et al.</i> , 2016	-EndoSequence BC Sealer (Brasseler, Savannah, GA, USA)	- MM sealer (Micro Mega, France)	Epoxy resin-based
		- Endoseal (Prevest Denpro, Jammu, India)	Zinc eugenol –based
		-ProRoot white MTA (Dentsply, Ballaigues Switzerland)	MTA- based
		- MM-MTA (Micro Mega, France)	MTA based
Colombo <i>et al.</i> , 2018	-BioRoot™ RCS (Septodont, Saint-Maur-des-Fosses, France)	-MTA Fillapex (Angelus Dental Solutions, Londrina, PR, Brazil)	MTA- based
	-TotalFill BC Sealer (FKG Dentaire SA La Chaux-de-Fonds Switzerland)	-Sealapex™ (Kerr, Orange, CA, U.S.A)	Polymeric calcium hydroxide-based
		-AH Plus (Dentsply-DeTrey Konstanz Germany)	Epoxy resin-based
		-EasySeal (Komet, Brasseler GmbH & Co., Lemgo, Germany)	Epoxy-resin sealer
Kapralos <i>et al.</i> , 2018	-TotalFill BC sealer (Brasseler USA, Savannah, GA, USA)	-AH Plus (Dentsply International Inc, York, PA, USA)	Epoxy resin-based
		-RoekoSeal (Coltene, Alstätten)	Silicon –based
		-Guttaflow 2(Whaledent, Alstätten, Switzerland)	Silicon-based

using two laboratory methods: ADT and DCT. The diameter of the inhibition zones in the first case was measured after 48 hours and the CFU in the second case were counting after 1, 24, 72 and 168 hours. The results showed that the inhibition zone of AH Plus™ sealer (10.31 ± 0.21 mm) was significantly larger than EndoSequence™ sealer (6.00 ± 0.03 mm; $p < 0.05$) (Candeiro *et al.*). A similar situation was observed in DCT, where Endosequence™ sealer had significantly smaller antibacterial activity than AH Plus™ sealer up to 1 hour of direct contact ($p < 0.05$). On the other hand, the results published by Singh *et al.* (2016) showed a highest inhibitory activity of EndoSequence™ (14.4 ± 1.49 mm), compared with other endodontic sealers: MM-MTA™ (14.3 ± 0.47 mm) and ProRoot™ MTA (13.9 ± 1.16 mm). The authors suggest that this effect may be attribute to the high pH released by this material (Singh *et al.*). However, there were not found statistically significant differences among these materials ($p > 0.05$). Moreover, Endoseal™ (0 mm) and MM–seal™ (0 mm) did not demonstrated inhibitory effect over *Enterococcus faecalis* (Singh *et al.*).

Similar results than observed by Candeiro *et al.* are published by Colombo *et al.* These authors, using ADT method, observed that all root canal sealers showed antibacterial activity except TotalFill™ sealer. In this reseach was included other bioceramic sealer, BioRoot™. It is a bioactive mineral based on tricalcium silicate, zirconium oxide and Povidone and has been determined that has an acceptable physico-chemical

properties. BioRoot™ (0.2 ± 0.05 mm), MTA Fillapex™ (0.3 ± 0.02 mm) and Sealapex™ (0.2 ± 0.04) presented the lowest antibacterial activity compared with AH Plus™ sealer (1.20 ± 0.15 mm) and EasySeal™ sealer (8.00 ± 1.41 mm). However, when using DCT method, for every contact time included in the methodology used (6 minutes, 15 minutes and 60 minutes) TotalFill™ has bactericidal effect against *Enterococcus faecalis* (Colombo *et al.*). Results published by Kapralos *et al.* showed that AH Plus™ had high antibacterial activity, however it was lost after 24 hours. TotalFill™ showed marked antibacterial effect on planktonic bacteria up to 7 days after setting and Guttaflow2™ and RoekoSeal™ had no antibacterial activity (Kapralos *et al.*). This authors used different conditions to examine whether the presence of water can affect the antibacterial properties of this bioceramic sealer (Kapralos *et al.*). No differences were observed in bacterial survival with or without storage in water. This evidences indicate that the high value of the initial pH of the endodontic sealers may not be the only factor to explain their antibacterial activity.

DISCUSSION

The use of root canal sealers materials with antibacterial activity is considered beneficial (Zhang *et al.*). A root canal sealer with antimicrobial properties may increase the success in endodontics because it

can contribute in the elimination of residual microorganisms or new microorganisms that can invade the root canal later through microleakage (Al-Haddad & Che Ab Aziz). Studies have been previously performed to assess the antimicrobial activity of different endodontic sealers. However, results of this literature review determined that there is no information available regarding the antimicrobial properties of bioceramic endodontic sealers in general (Zhang *et al.*). For these reasons was carried out a systematic review for this type of endodontic root canal sealer. The bacterium chosen for the study was *Enterococcus faecalis*, for several reasons (Du *et al.*, 2015). Within the characteristics of *Enterococcus faecalis*, could be mentioned that it is a part of the normal oral flora in humans, have been reported that is frequently found in mixed infections, it is one of the microorganisms most commonly isolated from the root canal, usually in secondary infections (Nair *et al.*, 2011), and has been strongly associated with persistent periapical infections and endodontic treatment failures (Rodríguez-Niklitschek & Oporto; Oporto *et al.*). The antimicrobial activity of bioceramic root canal sealers was evaluated using *in vitro* tests reported in the preceding literature. The effect of endodontic sealers on microbial viability has been examined by ADT, DCT and MDCT; ADT is the primary method for determining the antimicrobial activity of medical substances such as endodontic sealers. ADT is a qualitative method, easy to perform and simple (Balouiri *et al.*, 2016; Dwivedi *et al.*, 2017); it is one of the classic techniques of microbiology (Sandle, 2016), and is still commonly used because of its convenience, efficiency, and cost. This is probably one of the most used methods for determining antimicrobial resistance worldwide (Sandle). However, recent studies have demonstrated that there is an important variability in the diffusion capacity of different root canal sealers into the culture media, due to the physical and chemical characteristics of certain components of specific sealers. Thus, the use of ADT is not a method recommended for antimicrobial analysis of endodontic sealers (Kapralos *et al.*; Bukhari & Karabucak, 2019). DCT is other alternative, but this method does not consider the dentin structure and its composition and the biofilm formation (Bukhari & Karabucak). The MDCT is a quantitative and reproducible method, that simulates the contact between the endodontic sealer and the microorganism inside the root canal; it can be standardized to test insoluble materials, like endodontic sealers (Zhang *et al.*; Ghatole *et al.*, 2016).

The permanence of microorganisms or the recontamination of the root canals may be partly

attributed to their ability to penetrate into dentinal tubules where biofilms can be formed. Thus, inside the dentinal tubules the bacteria may be protected from the direct antibacterial effect of sealers (Wang *et al.*, 2014a). Although a variety of different models have been used to evaluate the effectiveness of endodontic sealers on planktonic form of microorganisms, the antibacterial activity of endodontic sealers against established biofilms has not been extensively studied (Baer & Maki). Although the endodontic sealers evaluated (Endosequence™, Bioroot™ and Totalfill™) showed antimicrobial activity against *Enterococcus faecalis* exhibited a decreasing antibacterial activity within a few hours or days after mixing (Wang *et al.*, 2014a).

CONCLUSION

Finally, the results of this systematic review showed the performance of bioceramic sealers evaluated *in vitro*, although it was found heterogeneous, depending of the laboratory method used. The antibacterial activity of these sealers seems to be effective at different degrees, for example, in the study performed by Colombo *et al.* the results of ADT showed lowest anti-bacterial activity for Bioroot™ compared to the others root canal sealers evaluated, although the limitation of this technique may cause false positive results. In conclusion, evaluated research showed that the bioceramic sealers may have various degrees of antimicrobial activity. With this information, it is not possible to make a conclusion about their comparative efficacy or recommend the use of one of them. Clinicians should be careful about the properties of endodontic bioceramic sealers until more evidence exists. Further research is required to compare the efficacy of bioceramic endodontic sealers, ideally *in vivo*. More reliable methods are needed that consider biofilm formation attached to dentinal root surfaces and the studies on humans are needed for make recommendations for the clinical practice.

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RESUMEN: En la actualidad, los cementos selladores biocerámicos son una opción ventajosa para la obturación de canales radiculares debido a sus múltiples propiedades. Existen varios estudios in vitro acerca de sus propiedades antimicrobianas, sin embargo, su potencial antimicrobiano, en comparación con otros tipos de cementos selladores endodónticos, no se está completamente claro. El propósito de la presente investigación fue realizar una revisión sistemática sobre las propiedades antimicrobianas de los cementos selladores biocerámicos disponibles en el mercado sobre *Enterococcus faecalis*, de acuerdo a la declaración PRISMA. Se realizó una búsqueda bibliográfica en cuatro bases de datos electrónicas: MEDLINE, ClinicalKey, ScienceDirect y EBSCO. Se incluyeron estudios in vitro, publicados en inglés, entre 2015 y 2019. La evaluación de los estudios seleccionados fue realizada por dos autores de forma independiente. Fueron incluidos cuatro estudios en la revisión. EndoSequence™ (Brasseler USA, Savannah, GA), también comercializado en el mercado como TotalFill™ (Brasseler USA, Savannah, GA), y BioRoot™ fueron los cementos selladores biocerámicos evaluados. El riesgo de sesgo se evaluó de acuerdo con la lista de verificación CONSORT modificada para estudios preclínicos. Los estudios seleccionados informaron actividad antimicrobiana positiva contra *E. faecalis* en los selladores biocerámicos evaluados. La menor actividad la presentó BioRoot™. Todos los estudios utilizaron una forma planctónica de *E. faecalis* y métodos de evaluación in vitro. La actividad antimicrobiana reportada varió en base al método de laboratorio utilizado, incluso para el mismo sellador, por lo que se sugiere la existencia de falsos negativos. Esta revisión sistemática identificó la necesidad de realizar más investigaciones sobre la actividad antimicrobiana de los selladores biocerámicos, idealmente con modelos que utilicen biopelículas bacterianas y estudios en humanos.

PALABRAS CLAVE: Agente antiinfeccioso, Bacteria, Biofilm, Endodoncia, *Enterococcus faecalis*, Sellador del conducto radicular.

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