The Saudi Dental Journal (2023) **35**, 769–779

King Saud University

The Saudi Dental Journal

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REVIEW ARTICLE

Methodologies used for testing the sealability of endodontic temporary fillings *in vitro*: A narrative review

Abdul Rahman MF ABDIN *, **Mouhammad H AL-TAYYAN**

Department of Operative Dentistry and Endodontics, Faculty of Dentistry, Damascus University, Damascus, Syria

Received 9 December 2022; revised 21 June 2023; accepted 2 July 2023
Available online 6 July 2023

KEYWORDS

Dental Leakage;
Dental Pulp Cavity;
Temporary Dental Restoration;
Endodontics

Abstract *Introduction:* The ability of the temporary filling to seal endodontic access cavities may be crucial for the success of endodontic treatment. Numerous *in vitro* studies have investigated the sealability of the temporary fillings used in endodontic treatments. However, *in vitro* sealability studies have been criticized for their inconsistent results and questionable clinical relevance. Some journals have imposed moratoriums on publishing such studies to encourage researchers to test their validity and clinical relevance. Since the implementation of this moratorium, little progress has been made in this field. To further encourage researchers to investigate the reliability of these studies, this review presents an overview of the methodologies of studies that examine the ability of temporary filling materials to seal the endodontic access cavity *in vitro* and discusses the criticisms of these studies in detail.

Materials and Methods: PubMed, Scopus, and Embase electronic databases were searched to identify studies that tested *in vitro* the ability of temporary filling materials to seal endodontic access cavities. Only original articles published in English between 01/01/1970 and 28/02/2022 were included.

Results: The search yielded 551 results. After removing duplicates and excluding studies that did not meet the inclusion criteria, 94 studies were included in this review.

* Corresponding author at: Department of Operative Dentistry and Endodontics, Faculty of Dentistry, Damascus University, Damascus, Syria.
E-mail addresses: abdulrahman.abdin@damascusuniversity.edu.sy (A.R.MF ABDIN), mouhmmad9.altayyan@damascusuniversity.edu.sy (M.H AL-TAYYAN).

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https://doi.org/10.1016/j.sdentj.2023.07.004

Conclusion: Although clinical studies may be the best way to test the performance of temporary fillings, the ethical importance of conducting preliminary *in vitro* studies is undeniable. It seems that questioning the reliability of *in vitro* sealability studies is not based on sufficient scientific evidence and that the inconsistencies in the results of these studies may be due to differences in the methodological and clinically relevant variables between them, rather than due to their unreliability.

Contents

1. Introduction

The endodontic access cavity (EAC) should be sealed between endodontic treatment sessions with a temporary filling (TF) that prevents the leakage of pathogenic microorganisms, their byproducts, and nutrients from the oral cavity and the leakage of intracanal medicaments outside the tooth (Rödig and Hülsmann, 2008). In addition, TF should be used to seal the EAC after completing the endodontic treatment until the time of placement of the permanent restoration.

A wide variety of TF materials are available on the market with various compositions and properties. Choosing a TF that provides a tight coronal seal is mandatory for the success of endodontic treatment (Rödig and Hülsmann, 2008).

Numerous *in vitro* studies have investigated the ability of TF materials to seal the EAC to help practitioners make evidence-based selections of TFs for use. However, these studies have been criticized for their inconsistent results and questionable clinical relevance (Wu and Wesselink, 1993).

In this narrative review, the methodologies of studies that examined the ability of TF materials to seal the EAC *in vitro* are reviewed, describing the testing techniques and tracers used, their advantages, disadvantages, and reliability, as well as the effects of differences in study design variables and clinically relevant variables on the results of these studies. In addition, criticisms of these studies are discussed in detail.

2. Materials and methods

2.1. Scope

This literature review focused on studies that have tested the sealability of TFs only. Studies that tested the sealability of interim restorations were beyond the scope of this review. Table 1 details the differences between interim restorations and TFs.

In addition, this review focused on studies that have tested the ability of TFs to seal EACs only. Studies related to restorative dentistry were beyond the scope of this review.

2.2. Search strategy

A literature search was conducted to identify papers that tested the ability of TF materials to seal EACs. The electronic databases PubMed, Scopus, and Embase were searched for articles published in English between 01/01/1970 and 28/02/2022. The search strategy used in PubMed is shown in Table 2.

Table 1 A detailed comparison between interim restorations and temporary fillings (Jensen et al., 2007; Patroni and Ferrari, 2004; Rödig and Hülsmann, 2008).

	Interim restorations	Temporary fillings
Usage	Build-up the tooth after removal of all caries and existing restorations. Used in teeth with missing lateral walls.	Seal the EAC, whether it is prepared within the dental tissues or within another interim or permanent restoration. The EAC should have no missing lateral walls, or only one missing lateral wall, with a pulp chamber that is deep enough to contain a sufficient amount of irrigants, and with the need for only short-term temporization of the cavity.
Duration	Placed before the initiation of endodontic treatment, and remains in place until the time of application of the final restoration after finishing the endodontic treatment.	Used to seal the tooth between treatment sessions. The TF remains in place for a shorter duration than the interim restoration.
Extent	Determined by the extent of the caries, the restorations and the cracks that existed previously. The EAC is prepared within the interim restoration, and is sealed between treatment sessions with a TF.	Determined by the extent of the EAC, i.e. by the anatomy of the pulp chamber and the root canals.

Table 2 The search strategy used in PubMed.

Search terms	
#1	((((((((((((((((temporary restorat*) OR (interim restorat*)) OR (intermediate restorat*)) OR (provisional restorat*)) OR (temporary filling*)) OR (interim filling*)) OR (intermediate filling*)) OR (provisional filling*)) OR (temporary seal*)) OR (interim seal*)) OR (intermediate seal*)) OR (provisional seal*)) OR (temporization)) OR (temporisation)) OR (provisionalization)) OR (provisionalisation)) OR (“coronal barrier*”)) OR (cavit)
#2	((((sealability) OR (sealing)) OR (leakage)) OR (microleakage)) OR (penetrat*)
#3	(dent*) OR (endodont*)
#4	(review[Publication Type]) OR (“case reports”[Publication Type])
#5	“*in vivo*”
#6	((((#1) AND (#2)) AND (#3)) NOT (#4)) NOT (#5)

Filters: Language: English, Date: 01/01/1970 – 28/02/2022.

The inclusion criteria were as follows: *in vitro* studies investigating the sealing ability of TFs in the EACs or cavities that reached the pulp chamber with no more than one lateral wall missing.

The rationale for including only EACs with no more than one missing lateral wall was that the current review focused only on TFs, which should ideally only be used in EACs with no more than one missing lateral wall (Table 1).

The exclusion criteria were as follows: (1) studies investigating the sealability of TFs when used as intra-orifice barriers or when used to seal post-prepared teeth or overdenture abutment teeth and (2) studies that did not include any commercially available TF material.

The search yielded 551 results. After removing the duplicates, 481 studies were screened. A total of 96 studies met the inclusion and exclusion criteria. Two of these studies were excluded because their full texts were unavailable (Etikan et al., 1987; Sevimay et al., 2004), thus 94 studies were included in this narrative review. The data from these studies were extracted and are presented in Supplemental Table 1. Table 3 summarizes the most relevant studies from Supplemental Table 1.

3. Review

3.1. ***In vitro*** *methodologies for the assessment of coronal sealing ability of TFs*

The following paragraphs present every method used in *in vitro* studies to assess the ability of TFs to seal the EAC.

3.1.1. Tracer penetration methods

These methodologies usually depend on evaluating the penetration of a tracer through the tested filling or between the filling and the dental tissues. The general principle of these methods is based on the hypothesis that the leakage of tracers through or around the fillings in extracted teeth *in vitro* is similar to the leakage of pathogenic microorganisms, their products, and nutrients through or around these fillings *in vivo* (Heintze, 2013).

Owing to the variety of tracers that can be used in tracer penetration studies (e.g., dyes, bacteria, and radioisotopes), there are numerous tracer penetration methods (summarized in Table 4).

3.1.1.1. Tracers. 3.1.1.1.1. Tracer's particle size. The particle size of the tracer used determines its degree of penetration and thus may affect the results of tracer penetration studies (Ahlberg et al., 1995). As it is necessary in endodontics to seal the root canal system against several types of pathogenic microorganisms, their toxins and nutrients (Wu and Wesselink, 1993), it is preferable to choose the tracer used such that the size of its particles is proportional to the size of the smallest pathogenic microorganisms, their toxins, or nutrients.

Tracers with larger particles (bacteria) overlook the leakage of bacterial byproducts and nutrients and the leakage of other pathogenic microorganisms that are smaller than bacteria, whereas tracers with particles that are too small (radioisotopes) may leak through dental tissues, which may lead to overestimation of leakage (Delivanis and Chapman, 1982; Friedman et al., 1986).

Table 3 The most relevant *in vitro* studies that investigated the coronal sealing ability of TFs in EACs*.

Study & Testing Technique (Tracer)	Tested Materials	Sample	Storage & Disinfection	Sealing Ability of TF Materials (Best > Worst)	Clinically Relevant Variables
Dye Penetration Method					
Teplitsky and Meimaris, 1988 Sample Immersion in Tracer/Longitudinal Sections with Filling Removal + Cotton Pellet Contamination (Methylene blue 10%)	Cavit, TERM	Permanent teeth	Water	Cavit > TERM	Thermal Changes; Time; Cavity Size & Design; Thickness of TFs; Endodontic Spacer; System & Curing Mode
Melton et al., 1990 Sample Immersion in Tracer/Clearing Technique (India ink)	Cavit, TERM	Permanent teeth	Deionized water with thymol	Cavit > TERM (both of them were through a PR)	Thermal Changes; Time; Cavity Size & Design; Applying TFs within IRs or PRs; Thickness of TFs; Endodontic Spacer; System & Curing Mode
Mayer and Eickholz, 1997 Sample Immersion in Tracer/Longitudinal Sections (Methylene blue 1%, Fuchsin red 1%)	Cavit, TERM, Kalsogen, IRM	Permanent teeth	1% thymol up to 30 days	Cavit = TERM > Kalsogen = IRM	Thermal Changes; Cyclic Loading; Time; Cavity Size & Design; Thickness of TFs; Endodontic Spacer; System & Curing Mode
Lai et al., 2007 Sample Immersion in Tracer/Filling Removal (Artificial saliva colored with 2% methylene blue)	Cavit, IRM, ZPC, copper bands cemented with ZPC	Permanent teeth		Cavit > IRM (P/L ratio 1:4) = ZPC (P/L ratio 1:4.4) = Copper bands cemented with ZPC (P/L ratio 1:4.4)	Thermal Changes; Time; Cavity Size & Design; Thickness of TFs; Endodontic Spacer; System & Curing Mode; Placement technique
Adnan and Khan, 2016 Sample Immersion in Tracer/Longitudinal Sections (Methylene blue 0.5%)	Cavit, IRM, Clip	Permanent teeth	Saline, after 24 h of immersion in 5.25% NaOCl	Clip = Cavit > IRM	Thermal Changes; Time; Cavity Size & Design; Applying TFs within IRs or PRs; Thickness of TFs; Endodontic Spacer; System & Curing Mode
Bacterial Penetration Method					
Barthel et al., 2001 Dual Chamber (*Staphylococcus epidermidis*)	Core buildup material (Clearfil), Core buildup material (CoreRestore), IRM, Ketac Fil, IRM over sticky wax, Ketac Fil over sticky wax	Permanent teeth	20% ethanol for various time periods	Core buildup material (Clearfil) = Core buildup material (CoreRestore) = IRM = Ketac Fil = IRM over sticky wax = Ketac Fil over sticky wax	Time; Cavity Size & Design; Thickness of TFs; System & Curing Mode; Double-layered TFs
Paranjpe et al., 2012 Sample Immersion in Tracer/Cotton Pellet or PTFE Tape Contamination (*Streptococcus gordonii*)	Cavit	Permanent teeth		No statistical analysis	Time; Cavity Size & Design; Thickness of TFs; Endodontic Spacer; System & Curing Mode
Abramovitz et al., 2013Dual Chamber (*Enterococcus faecalis*)	IRM, Coltosol, IRM with quaternary ammonium polyethyleneimine nanoparticles, Coltosol with quaternary ammonium polyethyleneimine nanoparticles	Cylindrical cavities in plexiglass plates		Coltosol with quaternary ammonium polyethyleneimine nanoparticles > IRM with quaternary ammonium polyethyleneimine nanoparticles > Coltosol > IRM	Thermal Changes; Time; Cavity Size & Design; Thickness of TFs; System & Curing Mode
Fluid Filtration Method					
Anderson et al., 1990 Fluid filtration Apparatus (Fluorescein 0.2%)	IRM with varying powder-to-liquid ratio	Permanent teeth			Thermal Changes; Time; Cavity Size & Design; Thickness of TFs; Endodontic Spacer; System & Curing Mode; Powder/Liquid Mixing Ratio

Table 3 (*continued*)

Study & Testing Technique (Tracer)	Tested Materials	Sample	Storage & Disinfection	Sealing Ability of TF Materials (Best > Worst)	Clinically Relevant Variables
Abramovitz et al., 2013 Fluid filtration Apparatus (Distilled water)	IRM, Coltosol, IRM with quaternary ammonium polyethyleneimine nanoparticles, Coltosol with quaternary ammonium polyethyleneimine nanoparticles	Cylindrical cavities in plexiglass plates		Coltosol with quaternary ammonium polyethyleneimine nanoparticles > Coltosol > IRM with quaternary ammonium polyethyleneimine nanoparticles > IRM	Thermal Changes; Time; Cavity Size & Design; Thickness of TFs; System & Curing Mode
Glucose Penetration Method					
Kim et al., 2015 Dual Chamber (Glucose)	Caviton, IRM	Permanent teeth		Caviton > IRM	Thermal Changes; Time; Cavity Size & Design; Thickness of TFs; System & Curing Mode
Radioisotope Penetration Method					
Marosky et al., 1977 Sample Immersion in Tracer/Longitudinal Sections (Ca^{45})	Temp-Seal, Cavit, ZOE, ZPC, IRM, PCC	Permanent teeth	Tap water except for a short period when they were placed in 1% NaOCl	Temp-Seal > Cavit > ZOE > ZPC > IRM > PCCNo statistical analysis	Thermal Changes; Time; Cavity Size & Design; System & Curing Mode
Friedman et al., 1986 Dual Chamber (Na^{22})	ZOE, IRM, Cavit G, Cavidentin	Permanent teeth	0.5% neomycin sulphate up to 10 weeks	IRM = ZOE > Cavit G = Cavidentin	Time; Cavity Size & Design; Thickness of TFs; System & Curing Mode
Electrochemical Method					
Jacquot et al., 1996 Electrochemical Method Apparatus (Sodium chloride)	Cavit, Cavit G, Cavit W, IRM	Permanent teeth		IRM (Capsules) > Cavit = Cavit W > Cavit G	Time; Cavity Size & Design; Thickness of TFs; System & Curing Mode
Quantitative Marginal Analysis					
Mayer and Eickholz, 1997 Quantitative marginal analysis	Cavit, TERM, Kalsogen, IRM	Permanent teeth	1% thymol up to 30 days	Cavit = TERM > Kalsogen = IRM	Thermal Changes; Cyclic Loading; Time; Cavity Size & Design; Thickness of TFs; Endodontic Spacer; System & Curing Mode

TF: temporary filling; EAC: Endodontic access cavity; ZOE: Zinc oxide eugenol; ZPC: Zinc phosphate cement; PCC: Polycarboxylate cement; NaOCl: Sodium hypochlorite; IR: Interim restoration; PR: Permanent restoration.

* See Supplemental Table 1 for the full version of this table.

Table 4 Tracer penetration methods used to test the coronal sealing ability of TFs in EACs.

Method	No of studies	Tracers	Testing Techniques
Dye Penetration	52	Methylene blue, Aniline blue, Alcian blue, Basic fuchsin, Eosin Y, Silver nitrate, India ink, Carbon black, Rhodamine B	Sample Immersion in Tracer
Bacterial Penetration	24	All bacteria present in natural human saliva, *Enterococcus faecalis*, *Proteus vulgaris*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus faecalis*, *Streptococcus gordonii*, *Streptococcus mutans*, *Streptococcus sanguis*	Dual Chamber Apparatus, Sample Immersion in Tracer
Fluid Filtration	10	Water, Dye solution	Fluid filtration Apparatus
Glucose Penetration	2	Glucose	Dual Chamber Apparatus
Radioisotope Penetration	4	Ca^{45}, S^{35}, Na^{22}, ^{3}H-thymidine	Dual Chamber Apparatus, Sample Immersion in Tracer
Electrochemical	3	Potassium chloride solution, Sodium chloride solution	Electrochemical Method Apparatus

Glucose and methylene blue dye seem to be clinically relevant tracers because glucose is a nutrient for bacteria, and methylene blue has a molecular size that is similar to that of bacterial metabolites and nutrients and less than the size of bacterial toxins (Kersten and Moorer, 1989; Matloff et al., 1982; Kim et al., 2015).

3.1.1.1.2. Tracer's viscosity. As saliva is more viscous than aqueous solutions of tracers, it has been claimed that leakage studies that use saliva as a tracer would better mimic oral conditions (Grossman, 1939). However, this claim is debated because the viscosity of saliva in the oral cavity decreases every now and then due to several normal daily habits, such as having hot or cold drinks, or using oral hygiene products that may contain agents that reduce surface tension (Parris and Kapsimalis, 1960). Thus, it seems that the use of aqueous solutions of tracers may simulate these conditions more closely.

3.1.1.1.3. Interactions with the tracer. Sometimes, interactions between the tracer and dental tissues or tested TF may occur, which may affect the results of the tracer penetration studies. For example, methylene blue dye loses its color when it comes in contact with materials such as amalgam, zinc oxide eugenol cement, Cavit (3M ESPE, Seefeld, Germany), and calcium hydroxide (Wu et al., 1998). In addition, some radioisotopes, such as Ca^{45} may interact with dental tissues or with the tested TFs (Delivanis and Chapman, 1982). Furthermore, glucose may interact with the tested TF (Shemesh et al., 2008). Besides, when the tested TF has antibacterial properties, bacteria should not be used as a tracer because the antibacterial properties of the TF would affect the results of the sealability test (Jafari and Jafari, 2017).

3.1.1.2. Testing techniques. 3.1.1.2.1. Sample Immersion in tracer. In this technique, after preparing the EACs and filling them with the tested TF, the outer surface of each sample tooth is completely sealed with coats of a sealing material (such as sticky wax), except for the area of the TF. Then, the samples are immersed in a tracer for a specified period and removed. The depth of the tracer penetration can then be evaluated in several ways, as detailed below.

Longitudinal sections technique

The most common methods for evaluating the depth of tracer penetration involve sectioning the samples longitudinally and measuring the depth of tracer penetration.

When using the dye penetration method, the penetration depth can be assessed under magnification (Adnan and Khan, 2016). When using the radioisotope penetration method, the ground surface of each section is placed on an emulsion of a dental X-ray film to produce an autoradiograph, and radioisotope penetration is assessed on these autoradiographs (Marosky et al., 1977).

The longitudinal sections method has been criticized for being destructive. In addition, because the axis of cutting the section is randomly selected, the deepest point of leakage may not appear in that section, which may lead to an underestimation of the depth of leakage (Camps and Pashley, 2003).

Filling removal technique

In this technique, the TF is removed and the depth of dye penetration is evaluated on all cavity walls under a microscope (Lai et al., 2007).

The main advantage of this technique is that dye penetration can be evaluated on all cavity walls. However, the leakage that may occur through the TF cannot be observed with this technique.

Clearing technique

In this technique, the samples are decalcified in nitric acid, washed thoroughly under running tap water, dehydrated with ethanol, and rendered transparent by placing them in a methyl salicylate solution (Melton et al., 1990). The depth of dye penetration can be evaluated on these cleared samples using a stereomicroscope.

This technique has the advantage of providing a three-dimensional view of what is inside the sample without being destructive (Jafari and Jafari, 2017). However, the main disadvantage of this method is that decalcification may be incomplete in some samples, which may adversely affect the accuracy of the results obtained from these samples (Melton et al., 1990). Additionally, this method may lead to partial dissolution of the dye (Oliver and Abbott, 2001).

Cotton pellet contamination with tracer

Some studies assess tracer penetration by observing the contamination of cotton pellets placed in the pulp chamber

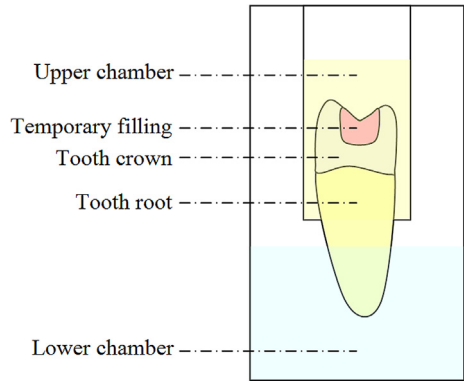

Fig. 1 The dual chamber apparatus for testing coronal leakage.

under the filling with the tracer (Paranjpe et al., 2012; Teplitsky and Meimaris, 1988).

One disadvantage of this technique is that it is impossible to identify and exclude samples in which leakage occurs through the enamel and dentin (in areas distinct from the TF) and reaches the cotton pellet (Teplitsky and Meimaris, 1988).

3.1.1.2.2. Dual chamber apparatus. In this technique, each sample tooth is tightly connected to two chambers: the upper chamber is connected to the coronal end of the tooth and the lower chamber is connected to the apical end of the tooth (Fig. 1). The tracer is placed in the upper chamber, and the leakage of tracer from the upper chamber through the tooth into the lower chamber is monitored.

A variation of this method involves placing the tracer inside the pulp chamber (instead of the upper chamber), placing each tooth sample into a vial (instead of the lower chamber), and monitoring the leakage of tracer out of the tooth into the vial (Friedman et al., 1986).

3.1.1.2.3. Fluid filtration apparatus. The roots of each tooth sample are discarded and an EAC is prepared and filled with the tested TF. The crown is cemented on a Plexiglas platform that is penetrated by an 18-gauge stainless-steel tube that extends into the pulp chamber. The stainless-steel tube is connected from its other side to the fluid filtration apparatus. The apparatus consists of a pressure reservoir containing a beaker. The reservoir is connected to a micropipette with polyethylene tubing, and additional tubing connects the micropipette to a microsyringe and to the stainless-steel tube. The beaker, tubes, micropipette, and microsyringe are all filled with water or dye solution. A constant gas pressure is applied to the reservoir, forcing the liquid to cross the gaps in the TF material and reach the tooth surface, which in turn causes the movement of a small bubble of air within the micropipette (Anderson et al., 1990). Leakage is measured by monitoring the rate of air bubble movement within the micropipette (Jafari and Jafari, 2017).

An advantage of this technique is that it permits measurement of leakage in each tooth sample before preparing the EAC; therefore, each sample can be used as its own control (Anderson et al., 1990).

However, this technique cannot measure the leakage that occurs through the filling, because the measurement period is very short (only a few minutes) (Jacquot et al., 1996).

3.1.1.2.4. Electrochemical method apparatus. For each tooth sample, the EAC is prepared and filled with the tested TF. After complete setting of the filling, an electrode is inserted from the apical foramen of the root into the inner surface of the TF. The outer surface of the tooth is completely sealed, except for the surface of the filling, and the crown of the tooth is immersed in an electrolyte (such as potassium chloride solution). Another electrode is immersed in the solution and an electric current is passed between the electrodes. The circuit is connected through the filling by the electrolyte that leaks around or through it, and the current is measured. The impedance is calculated according to Ohm's law from the ratio of the applied current to the measured current. The amount of electrolyte leakage is proportional to the degree of impedance produced (Jacquot et al., 1996).

Major discrepancies between the results of studies that used this technique to evaluate leakage can be observed. This can be explained by the differences in the details of the experimental model used, such as the type of electrode material and electrolyte used (Jafari and Jafari, 2017).

3.1.2. Quantitative marginal analysis with a scanning electron microscope (SEM)

The quantitative marginal analysis method depends on evaluating the marginal integrity of the filling *in vitro* to predict its sealability. This method is based on the hypothesis that the gaps between the filling and dental cavity walls form a pathway for the penetration of pathogenic microorganisms, their toxins and nutrients into the tooth, and that an evaluation of the marginal integrity of a filling might enable the researcher to predict its clinical performance (Heintze, 2013).

In this method, after preparation and filling of the cavities in the sample teeth, the teeth are imprinted, and impressions are cast for replica production. The replicas are then sprayed with gold for the SEM evaluation (Mayer and Eickholz, 1997).

Because the evaluation is performed on replicas, this method is nondestructive and allows repeated evaluations of the same sample (e.g., before and after cyclic loading) by producing replicas at each stage (Heintze, 2013; Mayer and Eickholz, 1997).

However, a disadvantage of this method is that the depths of the gaps along the cavity wall cannot be assessed (Mayer and Eickholz, 1997). In addition, this technique suffers from the inherent problem of relying on subjective assessments by researchers (Heintze, 2013).

3.2. Study design variables that may influence the results of in vitro sealability studies

3.2.1. Used samples

Supplemental Table 1 shows that there are differences between studies in the samples used, as well as in their storage duration and medium. To the best of our knowledge, the effects of these factors on the results of *in vitro* studies on the sealability of TFs are yet to be investigated. Therefore, further studies on this topic are required.

3.2.2. Statistical analysis

It can be noted from Supplemental Table 1 that in some studies, no statistical test was used. Also, a paper by Lucena et al. (2013) reported potential statistical errors in *in vitro* sealability studies. Therefore, attention must be paid to avoid drawing conclusions from studies with no or invalid statistical analyses.

Table 5 The *in vitro* studies that compared two methodologies of testing the coronal sealing ability of TFs in EACs.

Study	Tested Materials	Sample (Storage & Disinfection)	Clinically Relevant Variables	Method (Tracer)	Sealing Ability of TF Materials (Best > Worst)	Comparing Testing Methods
Mayer and Eickholz, 1997	Cavit, TERM, Kalsogen, IRM	Permanent teeth (1% thymol up to 30 days)	Thermal Changes; Cyclic Loading; Time; Cavity Size & Design; Thickness of TFs; Endodontic Spacer; System & Curing Mode	Dye penetration (Methylene blue 1%, Fuchsin red 1%)	Cavit = TERM > Kalsogen = IRM	The two methods yielded similar results
				Quantitative marginal analysis	Cavit = TERM > Kalsogen = IRM	
Abramovitz et al., 2013	IRM, Coltosol, IRM with quaternary ammonium polyethyleneimine nanoparticles, Coltosol with quaternary ammonium polyethyleneimine nanoparticles	Cylindrical cavities in plexiglass plates	Thermal Changes; Time; Cavity Size & Design; Thickness of TFs; System & Curing Mode	Fluid filtration (Distilled water)	Coltosol with quaternary ammonium polyethyleneimine nanoparticles > Coltosol > IRM with quaternary ammonium polyethyleneimine nanoparticles > IRM	The results of the fluid filtration method were related to the type of the TF material, while the results of the bacterial penetration method were related to the presence of an antibacterial agent. This is due to a known issue related to the bacterial penetration method which is the inability to distinguish the sealing ability of the TF material from its antibacterial efficacy
				Bacterial penetration (*Enterococcus faecalis*)	Coltosol with quaternary ammonium polyethyleneimine nanoparticles > IRM with quaternary ammonium polyethyleneimine nanoparticles > Coltosol > IRM	

3.3. Clinically relevant variables that may influence the coronal sealing ability of TFs

Because of the differences in the clinical cases that each *in vitro* study attempted to simulate, each study considered different clinically relevant variables (Supplemental Table 1). These differences in clinical variables between studies may have contributed to the apparent discrepancies in their results. Furthermore, the varying methods used to test these clinically relevant variables may have affected the results of the sealability studies. However, the methods for testing these variables are not detailed herein as they are beyond the scope of this review.

The clinical factors that were taken into consideration in TFs *in vitro* sealability studies were type of TF material; temperature changes; cyclic loading; time; intracanal medicaments; internal bleaching agents; application of TFs within dental tissues, interim restorations, or permanent restorations; size and design of the cavity; thickness of TFs; placement of an endodontic spacer under the TF; system and curing mode; powder/liquid mixing ratio of TFs; type of light-curing device; placement technique; and use of double-layered TFs.

3.4. Criticisms of in vitro leakage studies

Undertaking clinical studies may be the best way to evaluate the coronal sealing ability of TFs. However, conducting preliminary laboratory studies to test the sealability of TF materials may be ethically necessary before their use in clinical studies.

In vitro leakage studies have been criticized for the lack of standardized methods and the questionable clinical relevance of their results (Wu and Wesselink, 1993). These criticisms have reached the point that some endodontic journals decided to decline publishing them until their methods are standardized and the clinical significance of their results is known (De-Deus, 2012; Editorial Board of the Journal of Endodontics, 2007).

3.4.1. Lack of standardization of in vitro leakage studies' methods

Some researchers have criticized *in vitro* leakage studies, explaining that the lack of standardization of their methods may have resulted in inconsistent results (Raskin et al., 2001). However, considering that the clinical performance of TFs may vary according to the clinical conditions under which they are used, it should be noted that it is normal for the performance of the fillings in *in vitro* studies to vary according to the clinical conditions simulated in these studies (see paragraph 3.3).

As the abovementioned point may partially explain the inconsistency in the results of *in vitro* leakage studies, the effect of using different methodologies (dye penetration, bacterial penetration, etc.) in assessing leakage on the results may be undeniable (see Table 5). However, as can be noted from Table 5, the differences between the results of different methodologies for testing sealability, if any, can usually be explained and attributed to the known disadvantages of some methodologies.

3.4.2. Clinical relevance of in vitro leakage studies

The clinical relevance of *in vitro* leakage studies has been debated based on the differences between the results of *in vitro* leakage studies and those of some clinical outcome studies, such as those that tested the effects of coronal restoration quality on endodontic treatment outcomes (Ricucci et al., 2000; Torabinejad et al., 1990). However, many differences between these studies can be explained by the inaccuracy of clinical studies that rely on periapical radiographs for the assessment of periapical status instead of cone-beam computed tomography (CBCT) (Wu et al., 2009). In the abovementioned example, the results of the clinical studies that relied on CBCT corroborated the findings of the *in vitro* leakage studies (Gambarini et al., 2018).

The aforementioned debates were also based on two studies that did not find a correlation between the periapical status of sample teeth (prior to extraction) and *in vitro* dye penetration through the roots of these teeth (Oliver and Abbott, 2001; Susini et al., 2006). However, as these studies also assessed the periapical status on periapical radiographs instead of relying on CBCT, they may have provided inaccurate and misleading results (Wu et al., 2009).

Despite debates regarding the clinical relevance of *in vitro* leakage studies, some researchers have pointed out that these studies can be used to investigate the relative differences in the sealing abilities of different materials and techniques (Barthel et al., 2001; Oliver and Abbott, 2001).

4. Limitations

One limitation of this review is the inclusion of papers published in English only and the search of only three databases. Therefore, it is possible that relevant studies were not included in this review.

Another limitation is the inclusion of all *in vitro* studies with all methodological designs without excluding studies that have low methodological quality. This is because the goal of the current study was to review all the methodologies of the available studies and not their results.

5. Conclusions

From this literature review, the following conclusions could be drawn:

1. Clinical studies may be the best methods for testing the performance of TFs. However, the necessity for preliminary *in vitro* leakage studies before clinical studies is undeniable.
2. TFs should seal the EAC against pathogenic microorganisms, their byproducts, and nutrients, and not only against bacteria. Thus, *in vitro* leakage studies should use tracers that take all these factors into consideration, such as glucose or methylene blue dye.
3. Despite debates regarding their clinical relevance, *in vitro* leakage studies may provide information on the relative differences in sealability between the tested materials.
4. The inconsistencies in the results of *in vitro* leakage studies may not be due to their unreliability but rather to differences in the methodological variables and clinically relevant variables between them.

5. On one hand, there is insufficient evidence supporting the clinical relevance of *in vitro* leakage studies. On the other hand, all the evidence that claims to prove the unreliability of *in vitro* leakage studies seems to be unreliable. Thus, high-quality investigations of the reliability of *in vitro* leakage studies are required.
6. It is suggested that endodontic journals should consider high-quality *in vitro* preliminary TF sealability studies for publication instead of entirely rejecting all *in vitro* sealability studies.

CRediT authorship contribution statement

Abdul Rahman MF ABDIN: Conceptualization, Data curation, Investigation, Methodology, Resources, Visualization, Writing – original draft, Writing – review & editing. **Mouhammad H AL-TAYYAN:** Project administration, Supervision, Conceptualization, Methodology, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sdentj.2023.07.004.

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