Review Article

In vitro and *ex vivo* microbial leakage assessment in endodontics: A literature review

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Received: 20-07-16 Accepted: 12-10-16 Published: 12-12-16

Abstract

The aim of this study was to perform a literature review of published *in-vitro* and *ex-vivo* studies, which evaluated microbial leakage in endodontics in the past 10 years. A comprehensive electronic literature search was carried out in PubMed database for English articles published from 2005 to 2016 using the keywords "endodontics," "*in vitro*," "*ex vivo*," "microbial leakage," "microbial penetration," "saliva," "*Enterococcus faecalis*," "*E. faecalis*," "endodontic sealers," "temporary filling material," "apical plug," "mineral trioxide aggregate," and "MTA." The keywords were combined using Boolean operators AND/OR. Based on our search strategy, 33 relevant articles were included in the study. There are three main methods for assessment of bacterial microleakage, namely, (A) the dual-chamber leakage model, (B) detection of bacteria using a scanning electron microscope (SEM), and (C) polymerase chain reaction. All bacterial leakage models have some limitations and may yield different results compared to other microleakage evaluation techniques (i.e., dye penetration, fluid filtration, or electrochemical tests). The results of SEM correlated with those of microbial leakage test in most studies. Microbial leakage test using saliva better simulates the clinical setting for assessment of the leakage of single or mixed bacterial species.

Key words: *Dental leakage, endodontics, root canal obturation*

INTRODUCTION

The ultimate goal of endodontic treatment is to eliminate the diseased pulpal tissue from the root canal system, provide a suitable environment for healing, and prevent apical periodontitis. Microorganisms are the main cause of pulpal and periapical diseases.[1] Well-packed root canal filling material and a hermetic apical seal allowing no leakage are crucial for successful endodontic treatment.[2] Evidence shows that apical periodontitis is caused by intracanal bacteria.[3] Apical periodontitis is treated by chemomechanical cleaning and disinfection of the root canal system followed by filling of the root canal and providing apical and coronal seal to prevent reinfection.[4] However, many studies have reported bacterial penetration through the entire length of the root canal within a few days following root canal filling with gutta-percha, which indicates that a perfect seal is hard to achieve in endodontic therapy.[5,6]

In this regard, different methods have been designed for microbial leakage assessment in endodontics. The aim of this study was to evaluate the different techniques proposed for microbial leakage assessment in endodontics by reviewing the relevant articles published in the past 10 years.

A comprehensive electronic literature search was carried out in PubMed database for English articles published

Access this article online	
Quick Response Code:	Website: www.jispcd.org
	DOI: 10.4103/2231-0762.195516

How to cite this article: Savadkouhi ST, Bakhtiar H, Ardestani SE. *In vitro* and *ex vivo* microbial leakage assessment in endodontics: A literature review. J Int Soc Prevent Communit Dent 2016;6:509-16.

from 2005 to 2016 using the keywords "endodontics," "in vitro," "ex vivo," "microbial leakage," "microbial penetration," "saliva," "*Enterococcus faecalis*," "*E. faecalis*," "endodontic sealers," "temporary filling material," "apical plug," "mineral trioxide aggregate," and "MTA." The keywords were combined using Boolean operators AND/OR. Based on our search strategy, 33 relevant articles were included in the study [Table 1].

Search of the literature yielded 33 studies, which met our inclusion criteria. Information regarding the authors, titles, microbial leakage model used, and the results of the 33 studies are presented in Table 1.

Three main methods are available for assessment of bacterial microleakage, namely, (A) the dual-chamber leakage model, (B) detection of bacteria

Table 1: List of included studies on microbial leakage published from 2005 to 2016 in PubMed-indexed journals

Authors	Year	Title	Microbial leakage model	Result
Saberi *et al.*[7]	2016	*In-vitro* evaluation of coronal microbial leakage after post space tooth preparation	Dual-chamber leakage model using *Proteus mirabilis*	The leakage occurred within 7-21 days in the group without temporary filling whereas it occurred within 28-47 days in the group with temporary filling
Balto *et al.*[6]	2015	Obturation Techniques Allow Microbial Leakage Unless Protected	Dual-chamber leakage model using *Enterococcus faecalis*	No statistically significant difference was found between cold lateral compaction, continuous wave of condensation, or injectable gutta-percha
Amezcua *et al.*[8]	2015	Sealing ability of root-end filling materials	Dual-chamber leakage model using *Enterococcus faecalis+Staphylococcus aureus+Pseudomonas aeruginosa+Bacillus subtilis+Candida albicans*	Root-end fillings with Super-EBA or mineral trioxide aggregate had the lowest bacterial filtration and RealSeal showed the highest bacterial filtration
Kazemipoor *et al.*[9]	2014	Lack of correlation between microbial penetration method and electrochemical technique for assessment of leakage through the root canal fillings	Electrochemical microleakage test and dual-chamber leakage model using *Enterococcus faecalis*	The correlation between microbial penetration method and electrochemical technique was not statistically significant
Gomes *et al.*[10]	2013	Coronal microleakage of endodontically treated teeth with intracanal post exposed to fresh human saliva	Dual-chamber leakage model using fresh human saliva	90% microleakage in root canals after 24 hours in group 1 (root canals instrumented, obturated, and prepared to receive an intracanal post) and 70% microleakage in samples after 40 days in group 2 (root canals with cemented posts but without coronal sealing)
Navarro-escobar *et al.*[11]	2013	*Ex vivo* microbial leakage after using different final irrigation regimens with chlorhexidine	Dual-chamber leakage model using *Enterococcus faecalis*	EC40TM varnish showed the least leakage at 180 days, and was statistically similar to 2% chlorhexidine (CHX). No significant differences were observed between the group without final irrigation and 2% CHX group or 0.2% CHX plus 0.1% cetrimide

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Authors	Year	Title	Microbial leakage model	Result
Bakhtiar *et al.*[12]	2012	*In vitro* comparative study of the microbial leakage of one-step, thermafil and lateral condensation techniques	Dual-chamber leakage model using *Enterococcus faecalis*	Thermafil and one-step obturator can be advocated as effective obturation techniques for endodontic therapy
Maziar *et al.*[13]	2011	Comparing Microleakage in Root Canals Obturated with Nanosilver Coated Gutta-Percha to Standard Gutta-Percha by Two Different Methods	Dual-chamber leakage model using *Enterococcus faecalis*	There was 84% bacterial leakage in standard gutta-percha group and 76% in the nanosilver gutta-percha group
Shashidhar *et al.*[14]	2011	The comparison of microbial leakage in roots filled with Resilon and gutta-percha: An *in vitro* study	Dual-chamber leakage model using *Streptococcus mutans*	Resilon and Epiphany showed minimal leakage, which was significantly less than that of gutta-percha
Nawal *et al.*[15]	2011	A comparative evaluation of 3 root canal filling systems	Dual-chamber leakage model using *Enterococcus faecalis* and scanning electron microscopy	Resilon and GuttaFlow showed optimal sealing ability. AH Plus sealer along with gutta-percha showed poor sealing ability. Results of the scanning electron microscopy correlated with the results of microbial leakage test
Lodiene *et al.*[16]	2011	Sealing ability of mineral trioxide aggregate, glass ionomer cement and composite resin when repairing large furcal perforations	Dual-chamber leakage model using *Enterococcus faecalis* and scanning electron microscope	The percentage of leaking samples was significantly higher in composite resin than mineral trioxide aggregate and glass ionomer cement
Valadares *et al.*[17]	2011	The efficacy of a cervical barrier in preventing microleakage of *Enterococcus faecalis* in endodontically treated teeth	Dual-chamber leakage model using *Enterococcus faecalis*	Two or 3 mm of cervical barrier is effective in preventing microbial leakage
Aminsobhani *et al.*[18]	2010	Coronal Microleakage in Root Canals Obturated with Lateral Compaction, Warm Vertical Compaction and Guttaflow System	Dual-chamber leakage model using fresh human saliva	There were no significant differences among the experimental groups

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Authors	Year	Title	Microbial leakage model	Result
De Almeida-Gomes *et al.*[19]	2010	*Ex vivo* evaluation of coronal and apical microbial leakage of root canal--filled with gutta-percha or Resilon/Epiphany root canal filling material	Dual-chamber leakage model using fresh human saliva	There were no differences between the different filling materials (gutta-percha/ Grossman sealer and Resilon/Epiphany) and obturation techniques (lateral compaction and system B technique) in coronal or apical leakage
Drukteinis *et al.*[20]	2009	*In vitro* study of microbial leakage in roots filled with EndoREZ sealer/ EndoREZ Points and AH Plus sealer/conventional gutta-percha	Dual-chamber leakage model using fresh human saliva	Both types of root fillings-EndoREZ sealer/EndoREZ Points and AH Plus sealer/ gutta-percha points-showed microbial leakage with no statistically significant difference
Pitout *et al.*[21]	2009	Leakage of teeth root-filled with GuttaFlow and a single GP cone compared to lateral condensation and warm vertical condensation	Dye leakage and dual-chamber leakage model using *Enterococcus faecalis*	The microleakage of GuttaFlow using a single cone technique was similar to that of gutta-percha using lateral compaction and less than that of gutta-percha using vertical condensation
Jacobovitz *et al.*[22]	2009	Root canal filling with cements based on mineral aggregates: An *in vitro* analysis of bacterial microleakage	Dual-chamber leakage model using *Enterococcus faecalis*	Mineral trioxide aggregate and EndoBinder were efficient in sealing root canals
Salz *et al.*[23]	2009	Sealing properties of a new root canal sealer	Dual-chamber leakage model using *Streptococcus mutans*	Apexit Plus had better sealing ability in comparison with AH Plus
Fransen *et al.*[24]	2008	Comparative assessment of ActiV GP/glass ionomer sealer, Resilon/ Epiphany, and gutta-percha/AH plus obturation: A bacterial leakage study	Dual-chamber leakage model using *Enterococcus faecalis*	There were no statistically significant differences among the three obturation systems
Weston *et al.*[25]	2008	Comparison of preparation design and material thickness on microbial leakage through Cavit using a tooth model system	Dual-chamber leakage model using *Streptococcus mutans*	Four-mm thick Cavit prevented bacterial ingress for 2 weeks, however, microbial leakage may occur if the thickness is less than 3 mm or in a complex access preparation

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Authors	Year	Title	Microbial leakage model	Result
Pasqualini *et al.*[26]	2008	Microbial leakage of Gutta-Percha and Resilon root canal filling material: A comparative study using a new homogeneous assay for sequence detection	Polymerase chain reaction and then identification by the OCEAN technique for *Enterococcus faecalis*	Resilon showed greater microleakage and calcium hydroxide did not have a relevant impact on the quality of the apical seal
Hollanda *et al.*[27]	2008	Sealing ability of three commercial resin-based endodontic sealers	Dual-chamber leakage model using a mixture of bacterial markers	No statistically significant difference between Sealer 26 and AH Plus, although both materials differed significantly from Resilon/ Epiphany, which took less time to display microbial leakage
Ghoddusi *et al.*[28]	2007	An evaluation of microbial leakage after using MTAD as a final irrigation	Dual-chamber leakage model using *Streptococcus mutans*	It took longer for bacteria to penetrate when either EDTA or MTAD was used for smear layer removal. The root canals filled with AH Plus showed significantly longer resistance to bacterial penetration than canals filled with Rickert
Fathi *et al.*[29]	2007	An *in vitro* comparison of bacterial leakage of three common restorative materials used as an intracoronal barrier	Dual-chamber leakage model using *Enterococcus faecalis*	No significant difference was found between the Ketac-Cem, Clearfil Protect Bond/Clearfil AP-X and Maxcem
Monticelli *et al.*[30]	2007	Efficacy of two contemporary single-cone filling techniques in preventing bacterial leakage	Dual-chamber leakage model using *Streptococcus mutans*	Warm vertical condensation with gutta-percha/AH Plus appears to be more effective in minimizing bacterial leakage than single-cone technique with ActiV GP or Gutta-Flow
Chogle *et al.*[31]	2007	Intracanal assessment of mineral trioxide aggregate setting and sealing properties	Dual-chamber leakage model using *Enterococcus faecalis*	Apical moisture may affect the setting time or sealing ability of mineral trioxide aggregate
Munoz *et al.*[32]	2007	Microbial leakage of Enterococcus faecalis after post space preparation in teeth filled *in vivo* with RealSeal versus Gutta-percha	Dual-chamber leakage model using *Enterococcus faecalis*	No statistically significant difference existed in the microleakage of teeth filled with RealSeal compared with gutta-percha when post space was prepared

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Authors	Year	Title	Microbial leakage model	Result
Zehnder *et al.*[33]	2007	Prevention of bacterial leakage through instrumented root canals by bioactive glass S53P4 and calcium hydroxide suspensions *in vitro*	Dual-chamber leakage model using *Enterococcus faecalis* and scanning electron microscope	The bioactive glass material under investigation could not prevent the contamination of instrumented root canals
Karagenc *et al.*[34]	2006	A comparison of four different microleakage tests for assessment of leakage of root canal fillings	Fluid filtration, electrochemical, dye penetration, and bacterial leakage tests	There was a poor correlation among various methods for evaluation of hydraulic leakage
Celik *et al.*[35]	2006	Bacterial microleakage of barrier materials in obturated root canals	Dual-chamber leakage model using *Staphylococcus epidermidis*	Glass ionomer cement leaked significantly less when compared with flowable composite resin
Wang *et al.*[36]	2006	Effect of intracanal medicament on the sealing ability of root canals filled with Resilon	Dual-chamber leakage model using *Streptococcus mutans*	Calcium hydroxide did not adversely affect the seal of the root-canal system filled with Resilon
Yucel *et al.*[37]	2006	Effects of different root canal obturation techniques on bacterial penetration	Dual-chamber leakage model using *Enterococcus faecalis*	There was no significant difference between System B and lateral compaction technique at 60 days
Balto *et al.*[38]	2005	Microbial leakage of Cavit, IRM, and Temp Bond in post-prepared root canals using two methods of gutta-percha removal: An *in vitro* study	Dual-chamber leakage model using *Enterococcus faecalis*	Peeso-reamer yielded less leakage compared to using a hot plugger during the 30-day experimental period

using a scanning electron microscope (SEM), and (C) polymerase chain reaction (PCR). In the dual-chamber leakage model, there is a split chamber with a connection path through the root canal of the teeth fixed at the center. The upper chamber contains bacterial species cultured in brain heart infusion broth and the lower chamber contains the brain heart infusion broth. The entire root is covered with a sealing material while the root tip (apex) is left uncovered. In case of occurrence of bacterial leakage, the culture medium in the lower chamber becomes turbid.[17,23,27] In the SEM and PCR techniques, bacteria can be directly visualized or detected in the root canal or dentinal tubules.[26,33] Karagenc *et al*.[34] reported a poor correlation between the results of microbial leakage test and fluid filtration, electrochemical test, and dye penetration. Nawal *et al*.[15] showed that the results of SEM correlated with those of microbial leakage test. In the PCR method, DNA extracted from the specimens is amplified and then identified by the OCEAN technique.[16]

In the dual-chamber or split chamber model, the upper chamber may contain a single species (*E. faecalis*, *S. mutans*, *P. mirabilis*, or *S. epidermidis*),[7,17,23,35] multiple species,[8,27] or saliva.[10,18] Timpawat *et al*.[39] demonstrated that bacterial leakage model (mainly coronal) better simulated the clinical and biological setting than the dye penetration method. According to their study, most endodontic cements have adequate antibacterial activity to stop the ingress of bacteria. Microbial leakage studies cannot estimate the time of occurrence of periradicular infection because it

depends on several factors such as the virulence of microorganisms, defense capacity of the periradicular tissues, nutritional status, and bacterial interactions. However, chronic or acute infections may occur when microorganisms are present at the periapex.[40,41] The usage of human saliva is advantageous because it highly simulates the clinical setting. However, it cannot simulate the alterations in the oral environment such as thermal changes or the effect of dietary regimen on the salivary flow.[10,18,19] Verissimo *et al*.[42] showed that the evaluation of coronal leakage by use of bacteria provided more biologically significant and clinically relevant data than other methods.

Assessment of the sealing ability of gutta-percha obturation using saliva leakage method is based on the activity of salivary hydrolytic enzymes and their ability to break the seal.[18] Microbial products cause disintegration of gutta-percha and compromise the adaptation of gutta-percha to root canal walls, thus impairing the seal. In a study by Maniglia-Ferreira *et al*., decomposition and destruction of polyisoprene (the main substance of gutta-percha) produced high amounts of carboxyl and hydroxyl radicals during thermomechanical compaction and thermoplastic techniques, which resulted in molecular weight reduction and a decrease in the stability and sealing ability of the filling material and increased coronal microleakage.[43] In this review study, we found 31 studies that used split chamber technique (25 single species, 2 multiple species, and 4 saliva), of which 3 studies had used SEM and 1 study had used PCR technology.

CONCLUSION

All bacterial leakage evaluation techniques have some limitations, and may yield different results compared to other microleakage assessment methods (i.e., dye penetration, fluid filtration, or electrochemical tests). In most reviewed studies, the results of SEM correlated with those of the microbial leakage test. Microbial leakage test using saliva better simulates the clinical setting in assessment of leakage of single or mixed bacterial species. The greatest advantage of the PCR technique is its high specificity for detection of target microorganisms and decreasing the false positive results, which refer to the presence of residual bacteria within the root canal system before obturation.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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