Original Article

Assessment of Antibacterial Activity of Five Endodontic Sealing Materials Against *Enterococcus Faecalis*

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Abstract

Objective: Residual and recurrence infection can be prevented by a root canal sealer that possesses good antibacterial activity, which contributes to the success of endodontic therapy. This in-vitro study conducted to evaluate the antibacterial activity of five endodontic sealers (MTA fillapex, HS Mxima MTA, Endosequence BC sealer, BioRoot RCS, and AH plus) against *Enterococcus faecalis* using agar diffusion test.

Methods: Freshly mixed sealers were placed into prepared wells on agar plates. The diameter of the inhibition zone was measured after incubation for 24 hours. The data were analyzed statistically by ANOVA and LSD at a 0.05 significance.

Results: All tested samples had an antimicrobial effect against *Enterococcus faecalis* after 24 hours. MTA fillapex had the greatest antimicrobial effect with an inhibition diameter of 12.77mm, followed by Endosequence BC. Finally, the weakest antimicrobial effect was related to the BioRoot RCS sealer with a mean zone of inhibition of 10.26mm.

Conclusions: The root canal sealers showed different antimicrobial activity. However, MTA fillapex was the most effective sealer against the organism tested.

Keywords: *Root canal sealers, Antibacterial activity, Enterococcus faecalis.*

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Introduction

Bacteria and bacterial byproducts are considered as the main etiological factors that cause pulpal diseases and periapical lesions[1,2]. The primary goal for any endodontic treatment is the elimination of microbial agents from infected root canal space by instrumentation, irrigation, and the obturation[3-6]. Gutta-percha had many limitations; for example, it didn't bond and had no antibacterial properties. The root canal sealer is used to overcome all these drawbacks and fill the space between gutta-percha and dentinal wall, at the same time sealing the canal[7-9].

Infection in the root canal is divided into primary and secondary infections. *E. faecalis* is commonly isolated from both primary and secondary infections, but its prevalence is higher in secondary infections[9-11].

Many studies have been performed to investigate and measure the antibacterial activity of different endodontic sealers, agar diffusion test (ADT) is one of the most frequently used tools in this field[12]. MTA Fillapex is a newly developed MTA-based sealer. The philosophy behind this type of sealer is calcium oxide inside the material that reacts with water and produces calcium hydroxide that causes elevation of pH and antibacterial activity[13-15]. AH Plus is an epoxy resin-based sealer that liberates formaldehyde during the chemical hardening process, this sealer is considered as an improvement of properties and has a suitable flow and very well sealing properties with a very high opacity[16].

Endosequence BC sealer is a bioceramic material delivered as a premixed paste in the syringe with delivery tips for intracanal delivery of the material. According to many studies and manufacturer instructions, it has a working time of 30 minutes and has antibacterial properties during its setting reaction as it has alkaline pH[17-19]. HS Maxima MTA Sealer is a bioceramic root canal sealer on an MTA base. It is biocompatible for tissue recovery without reactions. The working time is 23 minutes and setting time 130 minutes[20].

BioRoot RCS A new hydraulic cement, BioRoot RCS (BR), was marketed as a mineral root canal sealer. It was a new calcium silicate-based cement similar in composition to Biodentine, is designed to be placed in permanent and close contact with periodontal tissue. In general, such hydraulic cement has given good results in conservative therapies. However, because BR has greater fluidity, it may be more practical in these applications. Enabling appropriate cell proliferation, migration, adhesion, and mineralization is an essential quality for a biomaterial in dentin-healing applications[21].

This study aimed to evaluate and measure the antibacterial properties of five different newly available sealers (Maxima MTA, MTA Fillapex, Endosequence BC sealer, BioRoot RCS, and AH plus) against *E. faecalis* after 24 hours.

Patients and methods

The composition of materials used in this study is shown in Table 1. The antimicrobial study was performed for all the materials using the agar diffusion test.

For *E. faecalis* preparation, a swab was taken and cultured on blood and Macconky agar. One colony from blood agar was taken from blood agar and placed into a test tube, and turbidity was adjusted to 0.5 Mcfarland with the aid of DensiCHEK plus. The identification of E. faecalis was confirmed with VATEK 2.

After confirmation, the colony was subcultured into a blood agar. A sterile loop was used to transfer the bacteria to the blood agar platen. Ten Petri dish was prepared, and five wells (diameter: 5mm, depth: 2mm) were punched in agar and filled with freshly mixed sealers according to manufacturer's instructions. The wells were adjusted to create a safe distance from each other and the edges to not overlap the inhibition zones around the wells. For the entire procedure, working was done under Bioair Topsafe with continuous air ventilation and Bunsen burner that was turned on near the working area to prevent contamination of the testing components by airborne pollutions.

After 24 hrs. The plates were taken out, and samples on the plate were removed using a sterile tweezer. With the aid of Maozua digital microscope, an image was captured. Afterword, the image was transferred to image J software for measurement of the inhibition zone.

Statistical analysis

The collected data were analyzed using one-way ANOVA and LSD test; statistically, a significant difference was set at $P<0.05$.

Table 1: Composition and manufacturer of the evaluated root canal sealers.

Sealer	Composition	Manufacturer
MTA Fillapex	Natural Resin, Salicylate Resin, Diluting Resin, Bismuth Trioxide, Nanoparticulated Silica, Pigments, And MTA	Angelus, Londrina, PR, Brazil
HS Maxima MTA Sealer	Base Paste: Salicylate Resin, Natural Resin, Calcium Tungstate, Nanoparticulated Silica, Pigments; Catalyst Paste: Diluting Resin, Mineral Trioxide Aggregate, Nanoparticulated Silica, Pigments.	Henry Schein Inc. USA
Endosequence BC sealer	(injectable form) a premixed calcium phosphate silicate-based sealer.	Brasseler USA, Savannah, GA, USA
BioRoot RCS	Powder-based on tricalcium silicate, zirconium oxide, and excipients. Aqueous solution of calcium chloride and excipient.	Septodont, St. Maurdes-Fosse´s, France.
AH Plus	Epoxy resin, calcium tungstate, zirconium dioxide, aerosil, iron oxide, adamantane amine, N, N'-dibenzyl-5-oxa-non andiamine-1,9, coloring, TCD-Diamine, silicone oil.	Dentsply De Trey GmbH, Konstanz, Germany

Results

Antimicrobial activities were evaluated with the measurement of the inhibition zone that was formed around each disk of sealer. The mean value and standard deviation of zone inhibition for each type of sealer against *E. faecalis* are shown in Table 2.

All tested sealers had an antimicrobial effect against *E. faecalis*. According to the results of one-way ANOVA (Table 3), there was a significant difference between all groups. Further analysis performed by LSD. Table 4 shows all groups had a significant difference in inhibition zone.

All tested sealers had antimicrobial effect against *E. faecalis* using one-way ANOVA (Table 3). Further analysis performed by LSD as shown in Table 4 all groups had significant difference in inhibition zone.

Table 2: The mean values and standard deviations of inhibition zones provided by the tested materials.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	39.278	4	9.819	104.872	0.0001
Within Groups	4.213	45	0.094		
Total	43.491	49			

Table 3: Analysis of variance between the studied sealers

Sealer	N	Mean(mm)	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum
				Lower Bound	Upper Bound		
HS MTA Sealer	10	11.318	0.125	11.229	11.408	11.148	11.501
MTA Fillapex	10	12.772	0.154	12.661	12.882	12.562	12.984
BioRoot RCS	10	10.263	0.471	9.926	10.6	9.567	11.089
AH plus	10	11.018	0.3	10.803	11.233	10.5	11.325
Endosequence BC sealer	10	12.219	0.34	11.975	12.462	11.752	12.765

Discussion

It has been stated that many bacterial strains play an important role in the progression of pulpal diseases and endodontic treatment failure. According to many studies, *E. faecalis* had higher resistance in the root canal and considered as a potential cause for endodontic failure. Accordingly, *E. faecalis* was chosen in this study. On the other hand, antimicrobial activity of sealers may help to reduce microorganism's activity after chemomechanical preparation of the canal and three-dimensional obturation[22-25].

The Agar diffusion method is widely in use for investigation of the antibacterial activity of dental and medical materials[25-27]. This method allows sealers to be kept in direct contact with microorganisms, and sealers can eliminate bacteria in areas such as the root canal system. Results of this method are affected by many factors, w viscosity of agar gel, temperature, an equal bacterial particle size, and ionic concentration of culture medium and period for all studied specimens[25,29].

In the present study, the antimicrobial effect of five types of sealers was assessed. Three calcium silicate-based bioceramic sealer, one bioactive calcium phosphate-based, and one resin-based sealer. The results showed that MTA Fillapex and bioceramic premixed sealer had more antibacterial effects than other types of sealers with significant differences among them. This may be due to that MTA Fillapex, and endosequence had antibacterial activity against *E. faecalis* before and after setting, and the activity sustains for more than seven days[16-18]. The antibacterial activity of all sealers was due to ingredients of all sealers that contain calcium disilicate or calcium trisilicate with hydrophilicity that cause calcium hydroxide release and production of high pH[1,2,18].

Table 4: Summary of LSD Post Hoc analysis of the inhibition zone.

(I) Factor	(J) Factor	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
HS MTA Sealer	MTA Fillapex	-1.453*	0.136	0.0001	-1.729	-1.177
	BioRoot RCS	1.055*	0.136	0.0001	0.779	1.33
	AH plus	0.299*	0.136	0.034	0.024	0.575
	Endosequence BC sealer	-0.9*	0.136	0.0001	-1.176	-0.624
MTA Fillapex	HS MTA Sealer	1.453*	0.136	0.0001	1.177	1.729
	BioRoot RCS	2.508*	0.136	0.0001	2.233	2.784
	AH plus	1.753*	0.136	0.0001	1.477	2.029
	Endosequence BC sealer	0.553*	0.136	0.0001	0.277	0.828
BioRoot RCS	HS MTA Sealer	-1.055*	0.136	0.0001	-1.33	-0.779
	MTA Fillapex	-2.508*	0.136	0.0001	-2.784	-2.233
	AH plus	-0.755*	0.136	0.0001	-1.031	-0.479
	Endosequence BC sealer	-1.955*	0.136	0.0001	-2.231	-1.68
AH plus	HS MTA Sealer	-0.299*	0.136	0.034	-0.575	-0.024
	MTA Fillapex	-1.753*	0.136	0.0001	-2.029	-1.477
	BioRoot RCS	0.755*	0.136	0.0001	0.479	1.031
	Endosequence BC sealer	-1.2*	0.136	0.0001	-1.475	-0.924
Endosequence BC sealer	HS MTA Sealer	0.9*	0.136	0.0001	0.624	1.176
	MTA Fillapex	-0.553*	0.136	0.0001	-0.828	-0.277
	BioRoot RCS	1.955*	0.136	0.0001	1.68	2.231
	AH plus	1.2*	0.136	0.0001	0.924	1.475

* The mean difference is significant at the 0.05 level.

While in AH Plus sealer which is epoxy resin base sealer, the antibacterial activity may be attributed to bisphenol-A- diglycidyl ether and minimum amount of formalehyde release. Many previous studies stated that AH Plus decrease cell proliferation of bacteria and had strong antibacterial effect[3,10,13].

It is believed that bioceramic sealer provide antibacterial effect from the release of calcium hydroxide by-product, triggering a very high alkaline pH that is toxic to the bacteria[22,30]. The alkaline pH from MTA fillapex promotes elimination of bacteria such as *E. faecalis*, *in vitro*[30,31]. The present experiment showed that MTA fillapex unveiled immediate, potent antibacterial effect up to 24 h. This is in agreement to the result of *in vitro* study that reported a short antibacterial action of BC sealer against *E. faecalis,* using a modified direct contact test[22]. Increase of pH level from the release of calcium hydroxide at early stage of setting creates an unsuitable environment for bacterial growth[32].

Conclusions

To conclude, the present study showed that MTA Fillapex sealer to have the highest antimicrobial activity against *E. faecalis*, while calcium phosphate-based sealer showed the least antimicrobial activity. However, these assessments are based on an in vitro aerobic culture technique, which may not immediately reflect clinical efficacy in vivo. The results have to be corroborated on a biofilm model to reflect clinical efficacy better.

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