Original Article

Antibacterial activity of root canal sealers against established monospecies biofilm: An *in vitro* study

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Abstract

Endodontic treatment is primarily a combination of chemical as well as mechanical preparation of canal space which helps in the placement of a biocompatible material. The main purpose of endodontic treatment is to eradicate microorganisms from the infected root canal system and prevent recontamination. The principle constituents of an endodontic filling are core material "Gutta Percha" and "Endodontic Sealers." Endodontic sealers should ideally eliminate residual bacteria and prevent reinfection after chemomechanical treatment and obturation of the root canal. The aim of this study is to investigate the antimicrobial effect of four endodontic sealers against bacteria in biofilms commonly detected from persistent and secondary endodontic infections.

Keywords: Antibacterial activity; bacteria; biofilm; endodontic sealer

INTRODUCTION

Microorganisms and their products are the main etiological factors in dentinal, pulpal, and periapical pathosis. The central aim of the root canal treatment is the elimination of bacteria from the infected root canal and prevention of subsequent reinfection.[1-5]

This is mainly achieved by thorough irrigation and biomechanical preparation of the root canal. These procedures undoubtedly reduce the number of viable organisms in root canal, but the complex anatomy of root canal often makes complete debridement impossible.

The presence of bacteria in dentinal tubules and cementum even after treatment has been reported. Bacteria that survive even after thorough chemomechanical preparation of the root canal system, and the dentinal tubules may be a source of recurrent infection after conservative as well as surgical treatment.[5]

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Bacteria remaining after instrumentation can be prevented from reinfecting the root canal system by an interappointment dressing such as calcium hydroxide (Ca[OH]2). However, Ca(OH)2 does not consistently produce bacteria-free canals, may allow regrowth, and is not effective against all bacterial species found in root canal infections.[6]

Remaining microorganisms can also be eliminated or rendered harmless by entombing them through complete obturation with Gutta-percha points and sealer after chemomechanical cleaning and disinfection. Those microorganisms can be killed by the antibacterial activity of the sealer or release of Zn ions from the Gutta-percha points or by depriving them of nutrition or space to multiply.

Endodontic disease is a biofilm-mediated infection, and the primary aim in the management of endodontic disease is the elimination of bacterial biofilm from the root canal system. Biofilm is embedded in a self-made matrix of extracellular polymeric substances and is a mode of microbial growth where dynamic communities of interacting sessile cells are irreversibly attached to a solid substratum, as well as to each other. The most common endodontic infection is caused by the

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surface-associated growth of microorganisms. It is important to apply the biofilm concept to endodontic microbiology to understand the pathogenic potential of the root canal microbiota.[7]

Consequently, the use of root canal filling materials with antibacterial activity is considered beneficial in the effort to further reduce the number of remaining microorganisms and to eradicate the infection.

The antibacterial effect of endodontic sealers has most often studies using agar diffusion test. This method does not distinguish between microbiostatic and microbicidal properties of the material. The antimicrobial activity of the sealer indicated by this test is influenced by the solubility of the material in the medium.

On the contrary, the direct contact test (DCT) measures the effect of direct and close contact between the organisms and the material, regardless of the solubility and diffusibility.[8]

However, neither of these tests measures the antibacterial activity of materials on established biofilms. For biofilms, the modified DCT and membrane-restricted test can be performed.

The purpose of this study was to investigate and compare the antibacterial effect of the endodontic sealers Apexit Plus (calcium-based root canal sealer), CeraSeal-B (mineral trioxide aggregate [MTA]-based bioceramic sealer), Endoseal (ZoE-based sealer), and Sealmax-R (Resin-based sealer) against established biofilms. The susceptibility of the Gram-positive bacteria *Enterococcus faecalis*, *Streptococcus mutans*, *Staphylococcus epidermidis,* and *Staphylococcus aureus* is tested after their planktonic growth and after biofilm formation.

MATERIALS AND METHODS

In the present study, the root canal sealers tested are as follows Figure 1:

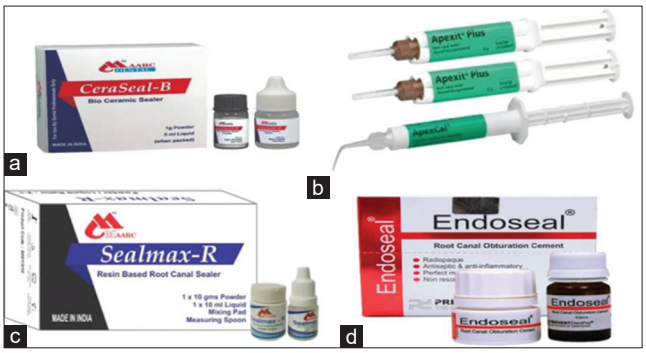


Figure 1: (a) Ceraseal-B. (b) Apexit plus. (c) Sealmax-R. (d) Endoseal

Pathogenic culture and media

Pathogenic bacterial cultures of *E. faecalis*, *S. aureus*, *S. epidermidis,* and *S. mutans* are procured from ATCC strains by HiMedia India Pvt Ltd. Aseptic culture is subcultured in sterile nutrient broth and incubated at 37°C for 24 h.

Antibacterial assay on established monospecies biofilm: Direct contact test

Sealer antimicrobial assay against bacterial biofilm DCT is used to investigate the antimicrobial activity of sealers according to Kapralos *et al*. Briefly, 10 µL from bacterial culture in brain heart infusion (OD450 = 0.1) is added into the test tubes [Figure 2a]. The tubes are then placed with the bottom up. Test tubes set are incubated at 37°C for 24 h. The tubes are then washed gently with phosphate-buffered saline (PBS) to remove unattached bacteria. Test tubes are added with crystal violet [Figure 2b]. For DCT, experimental sealers are mixed and directly applied onto the biofilm formed on the surface of the tubes [Figure 2c]. Biofilms with no exposure to sealer are used as positive control and tubes with sealers and no bacterial growth are used as a negative control. After 24 h of contact at 37°C, sealers are separated from tubes [Figure 2d]. Sealer samples are separately put in vials containing 10 mL PBS and vigorously vortexed. Serial dilution is done, and colonies of surviving bacteria are calculated for each material. The initial optical density (OD) in the microtiter plate is recorded using enzyme-linked immunosorbent assay reader at 450 nm [Figure 2e]. Colony-forming units are counted after incubation at 37°C in a 5% CO_2 supplemented atmosphere for 24 h for *S. epidermidis*, *S. aureus*, and *E. faecalis* and for 48 h for *S. mutans*.

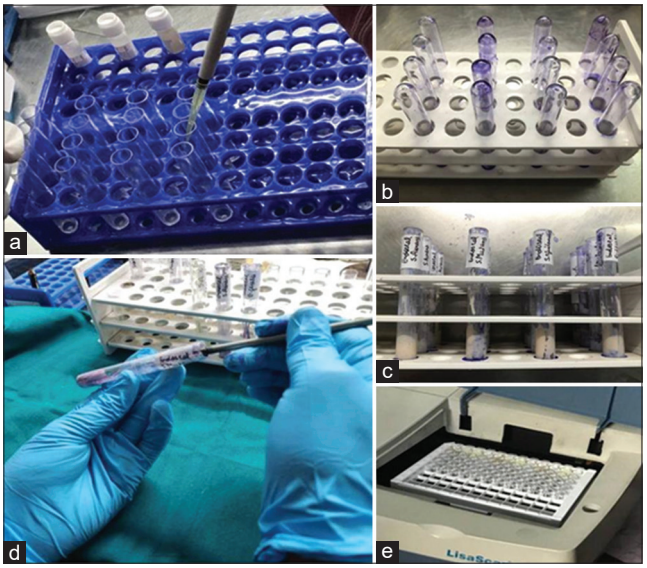


Figure 2: (a) Bacterial suspension is added. (b) Crystal violet is added to visualize the biofilms. (c) Sealer application on the biofilms. (d) Scraping of sealer. (e) Colony-forming unit is counted with spectrophotometer

Data are collected by recording OD, because as bacterial population increases, absorbance reading, i.e., OD given by spectrophotometer increases. Data are collected, plotted, and statistically analyzed.[7]

RESULTS

Endoseal showed a good carryover effect after serial dilution when checked overnight. Apexit Plus reduced bacterial survival significantly for all bacterial biofilms investigated. Sealmax-R reduced the bacterial colonies initially, but the antibacterial activity was lost gradually. CeraSeal-B had poor antibacterial activity against biofilm formed by any of the bacterial species investigated.

mean log CFU/ml of S. aureus bioflim testing after 1 hour by DCT					
S. aureus	ENDOSEAL	APEXIT PLUS	Seal max R	Ceraseal	control
DCT	0	0.347	1.983	3.996	4.256
mean log CFU/ml of S. epidermidis bioflim testing after 1 hour by DCT					
S.epidermidis	ENDOSEAL	APEXIT PLUS	Seal max R	Ceraseal	control
DCT	0.042	1.741	1.653	5.105	5.606
mean log CFU/ml of S. mutans bioflim testing after 1 hour by DCT					
S. mutans	ENDOSEAL	APEXIT PLUS	Seal max R	Ceraseal	control
DCT	2.106	1.856	1.658	4.808	7.991
mean log CFU/ml of E. fecalis bioflim testing after 1 hour by DCT					
E. fecalis	ENDOSEAL	APEXIT PLUS	Seal max R	Ceraseal	control
DCT	0.104	0.568	4.057	5.215	5.525

DISCUSSION

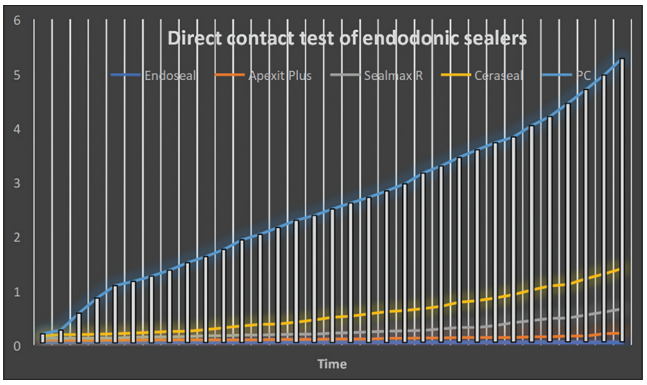
The presence of microorganisms has been observed in dentinal tubules and cementum even after the root canal treatment (Dalton *et al*. 1998,[9] Microbial persistence and growth in dentinal tubules, lateral canals, and apical ramifications have also been demonstrated (Love and Jenkinson 2002[10] and Torabinejad *et al*. 2018[11]).

A well-adapted sealer will only hinder the release of bacteria entrapped within the root canal system. However, for eradication of the remaining microorganisms, particularly when pulpal necrosis and apical periodontitis are present, the choice of a sealer with substantial antimicrobial activity could play an important role (Bergenholtz and Spångberg 2004[12]).

Therefore, it is important for us to use a sealer which has effective antimicrobial properties against these microorganisms to achieve a successful root canal therapy.

A biofilm model, i.e. DCT is performed to study the antibacterial effect of the sealers against monospecies biofilms.

A limited number of studies have investigated the antibacterial activity of endodontic sealers against already established biofilms.[7] In two studies, bovine dentin or human dentin was used as substrates to grow *E. faecalis*



Graph 1: Direct contact test of endodontic sealers

biofilms.[13] However, adherence of the tested material on dentin can lead to either a possible carryover effect or difficulties in retrieving all bacterial cells from the substrate.

Barros *et al*.[14] investigated the antibacterial activity of sealers for 30 min directly after mix. However, short contact time provides partial evidence of the antibacterial activity of the sealers against biofilms. Therefore, in the present study, we investigated the antibacterial activity against established biofilms for 24 h.

In the present study, Endoseal showed a good carryover effect after serial dilution when checked overnight [Graph 1]. Apexit Plus reduced bacterial survival significantly for all bacterial biofilms investigated. Sealmax-R reduced the bacterial colonies initially, but the antibacterial activity was lost gradually. CeraSeal-B had poor antibacterial activity against biofilm formed by any of the bacterial species investigated.

Our results are in agreement with the previous studies by Singh *et al*.,[15] which have shown that ZOE-based sealers possess a strong and persistent antimicrobial activity.

Many other studies have also reported the inhibitory activity of zinc oxide-based sealers (Rosa *et al*. 2022,[16] Barkhordar 1989,[17] Canalda and Pumarola, Pumarola *et al*. 1992,[18] Torabinejad *et al*. 2018,[11] and Leonardo *et al*. 2000[19]). This antibacterial action is probably related to the presence of eugenol (Leonardo *et al*. 2000[19]). Most antimicrobial activity of eugenol is conferred by their free hydroxyl groups , and in the past, some authors hypothesized that the hydroxyl group on eugenol is thought to get bind to proteins, preventing enzyme action.

Different mechanisms have been described to explain the activity of eugenol on bacterial cells. Primarily, it could act by the disruption of cytoplasmic membrane which increases membrane nonspecific permeability and affects the transport of ions and adenosine triphosphate. In a study it was demonstrated that eugenol was able to trigger

cell cytotoxicity due to the production of intracellular reactive oxygen species which induces the inhibition of the growth of cell, disruption of the cell membrane, and DNA damage, resulting in cell decomposition and death. Several biochemical mechanisms have been proposed to explain the cytotoxicity of eugenol and its utilization in restorations is targeted to prevent bacterial penetration.[20]

Thymol, which is present in this sealer, may affect the bacterial culture as well.[21,22] A study evaluated the antibacterial property of thymol, and the results showed that thyme essential oils had bacteriostatic activities against the microorganisms. Another evaluated the antimicrobial properties and mechanism of action of thymol, and the results of this investigation confirm that the main mechanism of action of thymol is the disruption of membrane integrity of bacterial cell.

Apexit Plus exhibited a good antibacterial activity. Root canal sealers with integrated (Ca[OH]2) have enhanced antibacterial activity.[23,24] The antimicrobial effect of this sealer stems from the release of hydroxide ions, which raise the pH to above 12.5.

The antibacterial activity of Sealmax-R-containing methanamine and bismuth oxide as its basic constituent was lost gradually. However, our results are not in agreement with any other previous study conducted on resin based sealers.

This is probably because of the different chemical composition of the resin-based sealers tested earlier. There has been no study published on the antibacterial property of Sealmax-R so far. In a study done by Rosa *et al*.,[16] it was found that highest short-term antibacterial activity of the tested sealers was shown in the poly-epoxide resin-based sealer due to its content of bisphenol A diglycidyl ether. Furthermore, in another study done by Park *et al*.[25] it was found that AH 26 which is composed mainly of epoxy resin was found more potent than any other sealers against *E. faecalis*.

CeraSeal-B showed poor antibacterial activity. These results are in agreement with studies done by Duarte *et al*.,[26] according to which pH values and calcium release provided by MTA-based root canal sealers were lower when compared with (Ca[OH]2)-based sealers.

However, the result is in contradiction with previous studies done by Mangat *et al*.,[27] which showed a potent antimicrobial property of bioceramic root canal sealers when compared with (Ca[OH]2)-based root canal sealer. This antibacterial activity is primarily related to the development of the nanostructure of the calcium aluminate particles which enhances the setting properties of bioceramic sealer and results in chemical composition and crystalline structure similar to bone and tooth apatite materials.

CONCLUSION

The aim of this *in vitro* study was to compare the antibacterial efficacy of four different root canal sealers against bacterial biofilms. According to the method, observations, and results, the following conclusion can be obtained. Among all the root canal sealers, EndoSeal showed a significant reduction in bacterial count followed by Apexit Plus, Sealmax-R, and CeraSeal-B in descending order.

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Conflicts of interest
There are no conflicts of interest.

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