To compare and evaluate the antimicrobial activity of three different root canal sealers: An In Vitro Study

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Abstract

Aim of the Study: The purpose of this study is to compare and analyze the antimicrobial activity of three different root canal sealers.

Materials and Methods: In this study, the antimicrobial activity of three different root canal sealers were compared against two strains of bacteria Enterococcus faecalis which is known to be common isolates of necrotic pulp and endodontic lesions. This test was done at various time intervals (1, 6, 15, and 60 min) using agar diffusion test and direct contact test.

Results: Bioceramic showed the best antimicrobial activity against E. faecalis among the three groups of sealers used. Moreover, the results were statistically analyzed.

Conclusion: Within the limitations of this study, it can be concluded that Bioceramic sealer showed the best antimicrobial activity followed by MTA Fillapex and Apexil.

Keywords: Antimicrobial activity; Apexil, Bioceramics; MTA

INTRODUCTION

Microorganisms are the primary pathological agents in endodontic disease, which alters the normal environment of the root canal by releasing endotoxins which re-infect the canal. For a successful root canal treatment, three-dimensional obturation of the canal system is required, following an impervious hermetic seal. Although endodontic obturation is mainly concerned with gutta-percha, still the endodontic sealers are used aiming to decrease the gap existing between the gutta-percha and the root canal walls.¹

To achieve an airtight seal, both obturating material and sealer play a pivot role. These sealers are used to obtain an impervious seal between the core material and the root canal wall. The morphology of the root canal is not always simple and straight. Anatomical deviations such as lateral canals, bifurcations, curved roots, apical ramifications, and deltas create a problem during apical sealing. Several authors have described that localized periodontal problems might be associated with infected root canal ramifications highlighting the importance of the endodontic sealer to flow into these irregularities. However, recent advances in root canal sealers have overcome this problem. These root canal sealers have a good sealing ability and antimicrobial activity to kill and eradicate the canal pathogens. The recent advances in root canal sealers are MTA and Bioceramics.

The properties of a root canal sealer have an impact on the final root filling. ADA specification no. 57 (1983) recommends the various physical properties of an endodontic sealer. The physical properties of various endodontic sealers have been extensively studied including the working time,
setting time, flow, film thickness, solubility, dimensional change, and radiopacity.\(^2\)

Different types of sealer have been introduced to endodontics, including those based on zinc oxide eugenol, calcium hydroxide, glass-ionomer cement, and a range of resins. Epoxy resin-type sealers have been used for many years. They showed higher bond strength to dentin than zinc oxide eugenol types and calcium hydroxide-based sealer. In recent years, different filling materials and sealers have been developed on the basis of dentin adhesion technology in an attempt to seal the root canal more effectively and to increase the fracture resistance of root-filled teeth. Furthermore, manufacturers have further incorporated adhesive dentistry in endodontics by introducing obturation systems with a specific focus on obtaining “monobloc” in which the core material, sealing agent, and root canal dentin form a single cohesive unit. The newer Bioceramic sealer is based on adhesive technology.\(^3\)

BC sealer is a recently introduced sealer composed of zirconium oxide, calcium silicates, calcium phosphate monobasic, calcium hydroxide, and various filling and thickening agents. The material is available in premixed calibrated syringes with intracanal tips. As a hydrophilic sealer, it utilizes moisture within the canal to complete the setting reaction and it does not shrink on the setting.\(^4,5\)

Thus, considering the various antimicrobial activity of an endodontic sealer and the recent advancement that is based on adhesive technology, three sealers were compared in our study: Apexit, MTA Fillapex, and Bioceramic.

**Aim of the study**
The aim of this study was to compare and evaluate the antimicrobial activity of three different root canal sealers, i.e., Apexit [Figure 1], MTA Fillapex [Figure 2], and Bioceramic [Figure 3]: An *in vitro* study.

**MATERIALS AND METHODS**

In this study, the antimicrobial activity of three different root canal sealers was compared against *Enterococcus faecalis* [Figure 4], which are known to be common isolates of necrotic pulp and endodontic lesions. This test was done at various time intervals using agar diffusion method.

Forty-five freshly extracted human teeth were used in this study. They were stored in formalin until use and cleaned of any debris and calculus with an ultrasonic scaler and used for the study purpose. Single root canal teeth were taken with no evidence of crack and fracture, root caries, resorption, and previous endodontic treatment. The root length was standardized at 12 mm and initial apical size no. > # 30 k file was used.

Each tooth was decoronated using a cylindrical diamond bur at high speed with water spray coolant. After enlarging the apical foramen up to k file size 30, root canals were instrumented with rotary file system up to the size F2, shaped, and cleaned using standard crown-down technique. The canals were then copiously irrigated with 5.25% sodium hypochlorite solution during shaping and cleaning procedure.

After final irrigation, canals were dried with paper points and obturated with laterally compaction gutta-percha technique with the above-mentioned three different sealers.
root canal sealers within their respective group – Group A: Bioceramic, Group B: MTA Fillapex, and Group C: Apexit.

After obturation, root-end sections were made from the apical area, considering 5 mm as a standard point on the surface of the root. From the root-end sections, the filling material was removed and added to the prepared inoculums.

Table 1 shows the tested sealers.

Preparation of the inoculums
Inoculum for the bacterial strain was prepared by picking up four to five colonies with the help of a circular, previously sterilized loop of 4 mm internal diameter and dissolving them into respective test tubes containing 5 ml of 0.85% saline solution. Petri dishes, 90 mm diameter, containing 4 mm thick Mueller-Hinton agar were used for the above bacterial strain. To ensure even distribution of the inoculums, the bacterial dilutions along with the sealer were then swabbed evenly onto freshly prepared agar plates. Three agar plates are taken, and in each plate, wells of 6 mm diameter were created with the help of previously fabricated and sterilized copper wells. The three wells in each plate were then filled with the three different based freshly mixed sealers.

Incubation
The inoculated plates with the sealers were kept for 2 h at room temperature to allow the prediffusion of the agents through the agar. The MH agar plates were incubated at 37°C for 24 h. A 0.05% of triphenyltetrazolium chloride was added for optimization. After solidification again, they are incubated at 37°C for 30 min. This allowed the differentiation between the areas of microbial growth (red areas) and diffusion zones. The plates for *E. faecalis* (facultative anaerobes) were read at 24 h, 48 h, 72 h, and lastly at 7 days for size of the zone of inhibition.[6]

**Measurement of zones of inhibition**
Growth inhibitory zones around each sealer were evident by lack of bacterial colonization (clearing of agar) adjacent to each sealer [Figure 5]. The most uniform diameter segment of the zone of inhibition was measured with an endodontic millimeter ruler. The measurements were taken for the inhibition zones. Wider zones of inhibition were interpreted to indicate greater antimicrobial activity of the involved sealers.

**RESULTS**
The result of this study showed that the mean antimicrobial activity was significantly higher in case of Bioceramic sealer as compared to MTA Fillapex and Apexit. The maximum antimicrobial activity was seen for Bioceramic at 24 h while the minimum antimicrobial activity was seen for Apexit after 7 days, which is shown in Table 2.

**Statistical analysis**
All statistical analysis was performed using the IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp SPSS 13 Chicago INC statistical software version. statistical software version. All the data were presented in a tabular and bar diagram form. The analysis of variance (ANOVA), *Post hoc* Bonferroni test, and paired *t*-test were performed to know the effects of each variable and to reveal the statistical significance. The confidence level of the study was proposed to be 95%; hence, *P* < 0.001 has been considered very highly significant.

ANOVA test was used for the three different sealers to check the antimicrobial activity against *E. faecalis*. Results revealed a highly significant (*P* < 0.001) difference among...
the sealer groups for their antimicrobial efficacy against *E. faecalis* at the four different time intervals.

Our study revealed the antibacterial efficacy of the three different test sealers against *E. faecalis* at various time intervals, i.e., 24 h, 48 h, 72 h, and 7 days. A statistically significant difference in antibacterial efficacy was seen for different sealers. It was seen that Bioceramic had a higher antibacterial efficacy as compared to all the other sealers. Results also showed that the antimicrobial activity of MTA Fillapex was almost similar at 24 and 48 h, but it was less than Bioceramic and more than Apexit, while Apexit showed the minimum antimicrobial efficacy at 7 days.

Table 3 shows the graphical presentation of the test sealers against *E. faecalis*.

**DISCUSSION**

One important characteristic of root filling material is antibacterial activity on microorganisms in the endodontic space. According to Grossman the ideal requirements for an endodontic sealer are: adequate consistency and adhesion to dentinal walls, adequate working time, capacity to produce a hermetic seal, easy handling, radiopacity, expansion at the time of set, biocompatibility, insolubility in tissue fluids, to allow retreatment of the canal, do not discolour dental tissues, no antigenic action, no mutagenic action and antibacterial action being one of the prerequisite of an ideal sealer. The antibacterial action may play a vital role in clinical situations of persistent/recurrent infection.\(^1\)

The chemical characteristics of the compounds found in root canal sealers may define important correlations with tissue tolerance, dentin bond strength, and antimicrobial properties.\(^7\)

Haddad and Che Ab Aziz in review stated that two methods commonly used to evaluate the antibacterial activity of bioceramic-based root canal sealers are the agar diffusion test and direct contact testing.\(^8\) In another study, Tiwari et al. also employed a similar methodology to evaluate the antimicrobial efficacy of two resin-based sealers.\(^9\)

The agar well diffusion method used in the present study is one of the most commonly employed techniques for the evaluation of antimicrobial activity. The prediffusion period, which consists of maintaining the inoculated culture medium at room temperature for 2 h, is an important step for demonstrating the antimicrobial activity of calcium hydroxide-based materials, MTA-based sealers, and Bioceramic sealer. The results of our study were in accordance with those obtained by Ribari et al., who employed similar methodology.\(^10\)

Our results revealed that all of the materials tested possessed antimicrobial activity, substantiated by the formation of growth inhibition zones in all the three sealers evaluated. The antimicrobial activity of calcium hydroxide-based materials (Apexit) may be related to ionization with subsequent release of hydroxide ions and an increase of pH levels, creating an unfavorable environment for microbial growth. It also has an anti-inflammatory property, low toxicity, and osteogenic repair potential.

The antibacterial effect of Apexit was also demonstrated by Sjogren et al. who put intracanal medication of calcium hydroxide in root canals of teeth with periapical pathology. They proved in their study the complete removal of *Actinobacillus actinomycetemcomitans*.\(^11\)

The antimicrobial activity of MTA was evaluated by Torabinejad et al., who demonstrated its effectiveness against some facultative bacteria. They observed an initial pH of 10.2 for MTA, rising to 12.5 in 3 h. It is known that pH levels in the order of 12.0 can inhibit most microorganisms, including resistant bacteria such as *E. faecalis*.\(^12\)
In another study, Morgental et al. who evaluated the antibacterial activity of MTA Fillapex and Endo CPM against 
*E. faecalis* concluded that the pH of Endo CPM suspension was greater than that of MTA Fillapex, however, bacterial 
inhibition zone was greater with MTA Fillapex.\[13\]

Bioceramic based sealer have showed a potent antimicrobial activity in this study. Its 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. Bioceramic sealer when used along with Gutta percha, it forms a hermetic seal in root canal 
that prevents the penetration of various microorganisms. The sealant is delivered in a pre-mixed syringe and does 
not require mixing as it can be applied directly into the canal using an intra-canal tip minimising wastage of 
material. The setting reaction of the Bioceramic cement is catalyzed by absorption of moisture present in the dentinal 
tubules contributing to hydration of cement and thereby forming a set mass that is radiopaque and biocompatible. 
Bioceramic sealers also don't shrink upon setting instead an expansion of .002% has been noted. Though 
manufacturers claim the flow rate of their sealers to meet the ISO requirement the literature does not support such 
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the flow rate of their sealers to meet the ISO requirement the literature does not support such claim. The use of a single-cone filling technique is recommended by studies. Moreover, it was indicated that bioceramic root 
canal filling materials can be used for filling root canals with or without gutta-percha points\[15,16\] and requires the 
presence of water to set and harden.\[17\]

Zhang et al. showed that Bioceramic-based sealer killed all bacteria within 2 min of contact. The authors proceed to 
explain that its potent antibacterial effect may be a combination of its high pH, hydrophilic nature, and its active calcium hydroxide diffusion.\[18\]

Bukhari and Karabucak concluded that EndoSequence BC Sealer exhibited significant antimicrobial capacity in the 
presence of dentin for up to 2 weeks on an 8-week-old *E. faecalis* biofilm in comparison with AH Plus sealer.\[19\]

The results of our study revealed that all the sealers tested possessed antimicrobial activity, as demonstrated by the 
formation of growth inhibition zones against *E. faecalis*. Bioceramic (smart bio paste) produced the largest zones 
of microbial growth inhibition against the bacteria, while Apexit showed the least antimicrobial activity. The efficacy 
of MTA Fillapex was less as compared to Bioceramic but greater than Apexit during the various time intervals 
mentioned in this study.

In a study by Kuga et al., the authors who compared the hydrogen ion and calcium releasing of MTA Fillapex and 
MTA-based formulations concluded that all materials showed alkaline pH and calcium releasing, with significantly 
lower values for MTA Fillapex sealer.\[20\]

In another study, by Gallusi et al. compared the antibacterial activity of first and latest generation bioceramic sealers on 
the elimination of *E. faecalis*. The three materials tested were Aureoseal (OGNA, Italy) (Aur) (first generation bioceramic 
sealer); EndoSequence BC sealer (Brasseler USA, Savannah, GA, USA) (EsBC) (latest generation bioceramic sealer); 
and EndoIDrox (OGNA, Italy) (Endx) (calcium hydroxide material). It was concluded that there has been no increase in the 
antibacterial efficacy of the latest generation bioceramic sealers compared to those of the first generation.\[21\]

**CONCLUSION**

On the basis of the results, observations, and statistical analysis, the following conclusion can be drawn from our 
study. (1) Bioceramic-based root canal sealer produced the largest inhibitory zones followed in decreasing 
order by Fillapex, MTA-based sealer, and least by calcium hydroxide-based root canal sealer. (2) All the tested sealers 
continued to show the inhibitory effect at 24 h, 48 h, 72 h, and 7 days, respectively.

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Nil.

**Conflicts of interest**

There are no conflicts of interest.

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