ORIGINAL RESEARCH

Stereomicroscopic Evaluation of the Apical Sealing Ability of Different Root Canal Sealers (Endoseal, Apexit, MTA Fillapex, Ceraseal) Using Diaphanization Technique

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Abstract

Introduction: Obturation of the root canal is the most critical step in endodontic treatment, which aims to provide a hermetic seal and prevent regrowth and entry of bacteria into the canal. To achieve this, many sealers are used in endodontics.

Aim: To evaluate the effectiveness of the apical seal obtained by different sealers used in conjunction with the single cone obturation technique using gutta-percha under the stereomicroscope.

Materials and methods: Extracted human single-rooted teeth were taken and decoronated at the cementoenamel junction. The access cavity was prepared, and biomechanical preparation was completed. The samples were randomly assigned to four groups consisting of 20 teeth each according to the root canal sealer used and categorized as group I, group II, group III, and group IV. Group I ($n = 20$)—Endoseal (Prevest DenPro), group II ($n = 20$)—Apexit Plus (Ivoclar Vivadent), group III ($n = 20$)—MTA-FillApex (Angelus), and group IV ($n = 20$)—Ceraseal (Meta Biomed). The teeth were immersed in Indian ink for 7 days and finally transferred to methyl salicylate for diaphanization. The extent of dye penetration was measured using the stereomicroscope. Statistical data analysis was performed using analysis of variance (ANOVA) and the Tukey test.

Results: Microleakage was seen in all the groups. Apical leakage was maximum for the Endoseal group, followed by MTA Fillapex, Apexit, and Ceraseal. Groups were compared by one-factor ANOVA, and the significance of the mean difference was measured using Tukey's honestly significant difference (HSD) and *post hoc* test. A statistically significant difference in the depth of dye penetration was observed among the groups ($F = 28.66$, $p < 0.001$).

Conclusion: It was concluded that there were statistically significant differences among the experimental groups. Ceraseal endodontic root canal sealer provided a significantly better apical seal, followed by Apexit and MTA Fillapex, whereas Endoseal showed the least sealing ability.

Keywords: Apexit, Apical microleakage, Ceraseal, Endoseal, MTA Fillapex.

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Introduction

Endodontic therapy aims to eliminate microbial existence, prevent reinfection of root canals, and seal the canals three-dimensionally to obtain a hermetic seal.[1] Obturation of canals is the final step in root canal treatment.[2] Thus, the success of the root canal depends on proper sealing from the apical foramen to the root canal orifice. Improper filling of canals causes reentry and regrowth of bacteria that do not favor periapical tissue and affect the treatment.[3] Improper filling also results in fluid movements into defects, which cause inflammatory reactions, compromising treatment success.[4] Gutta-percha, the most frequently used core material, fills root canals.[5]

Despite much research, no materials fulfill all the requirements to properly seal root canals. Apical leakage, influenced by sealer quality, smear layer, and variations in obturation procedures, is one of the primary reasons for the failure of root canal therapy.

Due to the intricate structure of canal anatomy, debris and pulpal remains can persist in areas inaccessible to irrigation solutions and equipment. These regions provide nourishment to the microbes residing in the canals, which can encourage bacterial growth and ultimately cause them to spread into periradicular areas through connections between the sealer and dentin.

Therefore, to achieve a three-dimensional, hermetic closure, it is imperative to utilize an excellent root canal sealer. To avoid gaps or voids at the sealer-dentin interface, it is also critical for the root canal sealers to improve adhesion between the gutta-percha and dentinal walls. Following obturation, an adequately sealed canal is necessary for a successful outcome of the endodontic procedure.

There are many techniques, including gas chromatography, bacterial permeability, clarification, and electrochemical approaches, to assess the sealing efficacy of root canal sealers.[6] The dye penetration test is the most accepted method for evaluating the sealing ability of sealers.

In endodontics, a wide variety of sealers are used. Sealers should have low consistency and good wettability to seal imperfections in

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root canals and fill the area between gutta-percha cones and root canal surfaces. Conventionally, endodontic sealers are categorized into different groups based on the key ingredients, which include zinc oxide eugenol (ZOE), $Ca(OH)_2$, MTA, and bioceramic.

Zinc oxide eugenol sealers have been accepted for root canal purposes for many years, whereas $Ca(OH)_2$-based sealers have demonstrated excellent sealing and biocompatibility, stimulating periapical tissues for healing.[7]

Traditionally, sealers have a few disadvantages: they contract after setting and wash out in the presence of tissue fluids, leaving voids that can lead to microleakage. MTA-based sealers have various properties that stimulate the growth of bone tissues. They are also water-soluble and adhere firmly to the dentinal wall, which is an excellent approach to eliminating microspaces.[8] Introduced >30 years ago, bioceramic-based sealers are predominant in medical and dental fields. They comprise a combination of calcium hydroxide, fillers, thickening agents, zirconium oxide, and calcium silicates.[9]

Limited research has been conducted to compare the microleakage of these sealers. The present study was intended to measure the sealing performance of four types of root canal sealers: ZOE, calcium hydroxide, MTA, and bioceramic.

Materials and Methods

The study was conducted in the Department of Pedodontics and Preventive Dentistry, IDS Bareilly, in collaboration with Oral Pathology and Microbiology, Bareilly, Uttar Pradesh. The study received approval from the ethical committee of IDS Bareilly.

Eighty caries-free, intact, single-rooted mature premolars were selected for the study after clinical and radiographic evaluation based on the inclusion criteria.

Exclusion criteria: hypoplastic teeth, teeth with any dental abnormality, cavities, or root fractures; teeth with either internal or external resorption; calcified canals; hunched roots; or dilacerated roots.

To achieve a consistent root length of 12 mm, eight teeth were decoronated at the cementoenamel junction (CEJ) using a carborundum disk. An endo access bur was used to open the access, and a size 15 K File (Main Inc.) was inserted into each root canal, extending beyond the apical foramen. The working length was calculated by deducting 1 mm from this length.

Biomechanical preparation was performed using a step-back technique with size 15 K Files up to size 40 K Files. Canals were replenished alternately with 2 mL of 3% NaOCl and normal saline during the procedure.

Grouping: The entire sample ($n = 80$) was split into four groups at random, with each group consisting of 20 teeth.

Group I ($n = 20$)—Endoseal (Prevest DenPro).
Group II ($n = 20$)—Apexit Plus (Ivoclar Vivadent).
Group III ($n = 20$)—MTA-FillApex (Angelus).
Group IV ($n = 20$)—Ceraseal (Meta Biomed).

Before the placement of the sealer, canals were dehydrated with absorbent paper points. The sealers were manipulated as stated by the manufacturer and filled into the prepared root canal space using a motor-driven size 30 lentulospiral at 300 rpm for 20 seconds. Canals were obturated with gutta-percha (single cone technique), and excess gutta-percha was cut off from the canal orifices using a heated instrument. After filling the root canals, the access was sealed with type II glass ionomer cement. All specimens were radiographed to check the obturation and stored at room temperature for 24 hours for complete sealer setting.

Two layers of nail varnish were applied to the tooth's surface, leaving the apical 2 mm uncovered to allow the dye to enter the canal solely through this area. Following a week in Indian ink, the samples were rinsed under running water, the nail varnish was removed with a surgical blade, and then the clearing process was performed.

Diaphanization technique: It consists of the following steps: decalcification, dehydration, and transparency.[11]

Samples were immersed in 5% nitric acid for 3 days, with daily acid changes and shaking three times a day. A pointed probe was then used to check for decalcification in the roots. Specimens were decalcified and prepared for the next step.

Roots were soaked in running water for 3 hours and then gradually immersed in increasing concentrations of ethyl alcohol for dehydration (Hong Yang Chemicals, China).

The samples were immersed in 80% alcohol for one night, followed by 3 hours in 90% alcohol and 3 hours in 100% alcohol.

The final step was transparency by submerging the specimens in methyl salicylate for 3 hours until the specimens were transparent and ready for evaluation. The clearing was achieved with methyl salicylate (S D Fine Chemical Ltd.).

The depth of dye penetration was examined under the stereomicroscope (Hamilton, Hamilton International SRL, Lazio, Italy) using a millimeter scale. Images were captured using Pro 4.6% software. Each specimen was photographed at 30× magnification with a digital camera fitted to the microscope. The depth of dye extent was graded according to Saunder's scoring criteria,[8] as shown in Table 1.

Analytical Statistics

The data were presented as mean ± standard error (SE) or standard error of the mean. One-factor analysis of variance (ANOVA) was used to compare the groups, and Tukey's honestly significant difference (HSD) *post hoc* test was used to determine the significance of the mean difference between the groups after Shapiro–Wilk's test and Levene's test had established the groups' homogeneity of variance. Statistical significance was defined as a two-tailed ($\alpha = 2$) $p < 0.05$. Statistical Package for the Social Sciences (SPSS) software (Windows version 22.0) was used for the analysis.

Results

Figure 1 exhibited apical dye penetration for each experimental material. The depth of dye penetration of four different root canal sealers or groups (group I, group II, group III, and group IV) is described in Table 2 and illustrated in Figure 2. The mean depths of dye penetration for group I, group II, group III, and group IV varied between 5–12, 1–8, 3–11, and 0–5 mm, respectively, with means (± SE) of 6.80 ± 0.46, 3.30 ± 0.47, 5.05 ± 0.43, and 1.50 ± 0.34 mm, respectively, and medians of 6, 3, 5, and 1 mm, respectively. The mean depth of dye penetration for group IV was the minimum, followed by group II, group III, and group I, which had the maximum (group IV < group II < group III < group I).

Table 1: Scoring criteria for apical leakage[8]

Degree of leakage	*Depth of penetration*
0	No leakage detected
1	<1 mm
2	1–1.5 mm
3	>1.5 mm

An ANOVA revealed significant differences in the mean depth of dye penetration among the four groups ($F = 28.66$, $p < 0.001$) (Table 3).

Additionally, in the intergroup comparison of mean depths of dye penetration, the Tukey test revealed that group II, group III, and group IV had significantly ($p < 0.05$ or $p < 0.001$) different and lower depths of dye penetration compared to group I (Table 4 and Figs 3 to 5).

Furthermore, it was found that group II and group IV had significantly ($p < 0.05$ or $p < 0.001$) different and lower values than group III. Moreover, Group IV was found to be significantly lower ($p < 0.05$) compared to group II.

Ceraseal (bioceramic root canal sealer) was found to have the minimum microleakage and is thus considered the best among the studied root canal sealers. Furthermore, it exhibited 77.9%, 54.5%, and 70.3% less mean depth of dye penetration than ZOE, calcium hydroxide, and MTA-based root canal sealers, respectively (Fig. 1).

Discussion

The main objective of root canal treatment is to thoroughly clean and shape the canals and achieve complete apical and coronal closure through three-dimensional obturation.[12]

Endodontic success is best achieved with conventional root canal therapy. Gutta-percha, in combination with a sealer, is the most commonly used technique for root filling. It is chosen for its ease of manipulation, minimal toxicity, minimal tissue irritability, and radio-opacity.[13–15]

Organisms that are present in the mouth or those that enter from outside can cause further inflammation. Therefore, it is vital to seal the canals adequately. Proper root canal cleaning cannot be achieved because of its complexity, and for sealing these canals precisely, adequate instrumentation and sealing are major requisites.[16,17]

Table 2: Depth of dye penetration (mm) of four groups

Group	*N*	*Depth of dye penetration (degree) (mean ± SE)*
Group I	20	126.18 ± 0.64
Group II	20	54.43 ± 0.63
Group III	20	104.75 ± 0.60
Group IV	20	73.78 ± 0.59

Depth of dye penetration of four groups were summarized in mean ± SE

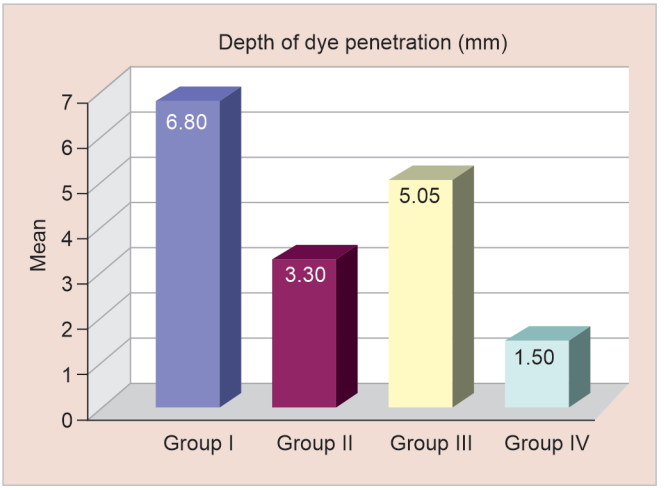

Fig. 2: Mean depth of dye penetration of four groups

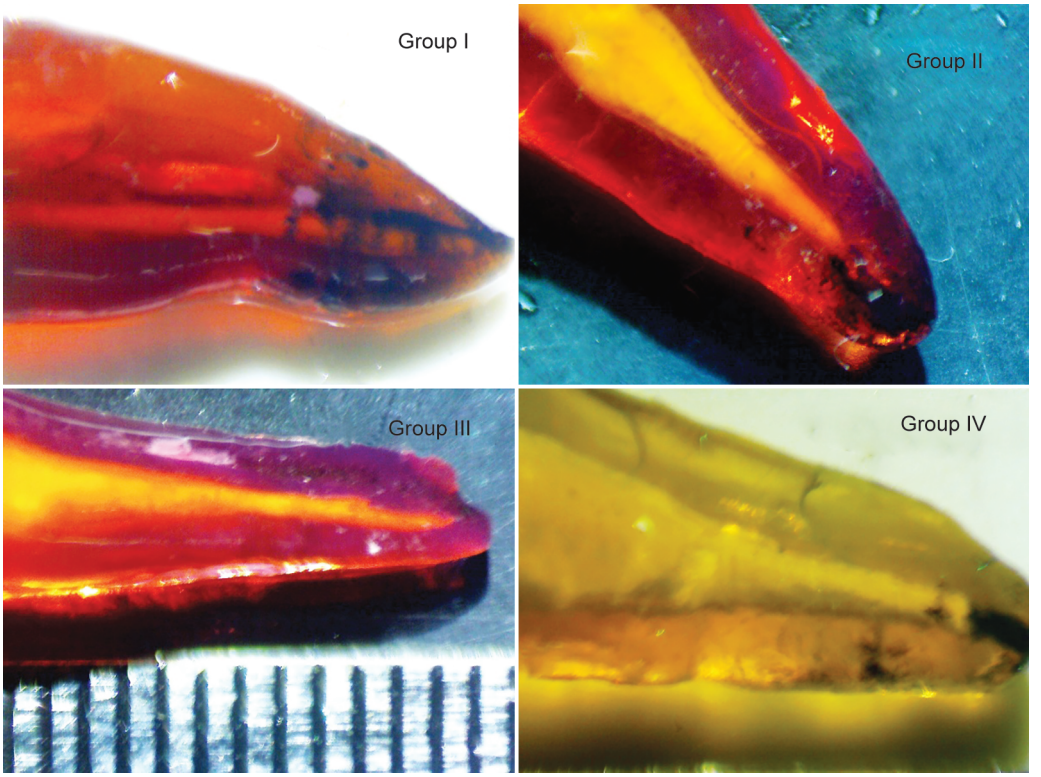

Fig. 1: Stereomicroscopic evaluation of decalcified samples and depth of dye penetration were measured at the apical region in groups I, II, III, and IV

Table 3: Comparison of mean depth of dye penetration (mm) among groups using ANOVA

Source of variation (SS)	*Sum of square (SS)*	*Degree of freedom (DF)*	*Mean square (MS)*	*F-value*	*p-value*
Between groups	311.50	3	103.80	28.66	<0.001
Residual	275.40	76	3.62		
Error	586.90	79	107.42		

F-value: ANOVA *F*-value

Table 4: Comparison (*p*-value) of difference in mean depth of dye penetration (mm) between groups by Tukey test

Comparison	*Mean diff.*	*q-value*	*p-value*	*95% CI of diff.*
Group I vs group II	3.50	8.22	$p < 0.001$	1.915 to 5.085
Group I vs group III	1.75	4.11	$p < 0.05$	0.165 to 3.335
Group I vs group IV	5.30	12.45	$p < 0.001$	3.715 to 6.885
Group II vs group III	–1.75	4.11	$p < 0.05$	–3.335 to –0.165
Group II vs group IV	1.80	4.23	$p < 0.05$	0.215 to 3.385

CI, confidence interval; diff., difference; *q*-value, Tukey test value

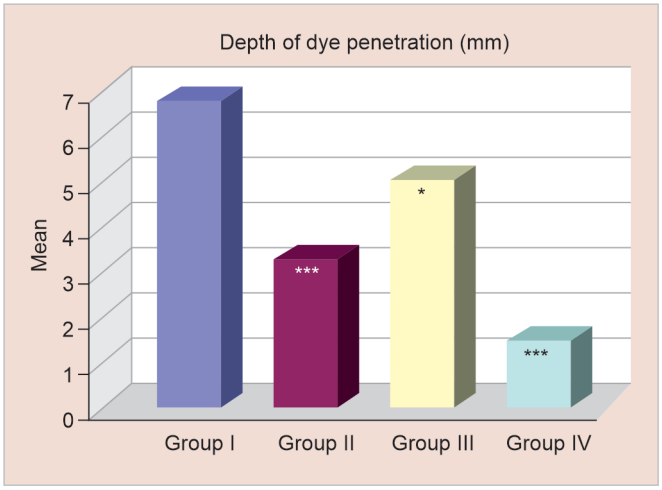

Fig. 3: Comparisons of difference in mean depth of dye penetration between four groups. *$p < 0.05$ as compared to group II

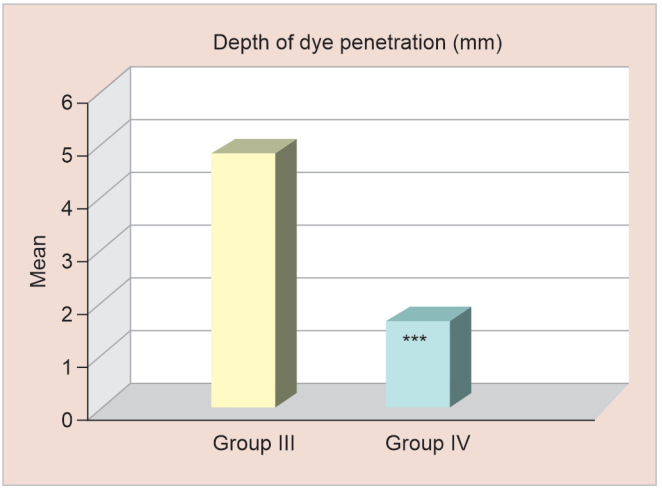

Fig. 5: Comparisons of difference in mean depth of dye penetration between two groups. ***$p < 0.001$ as compared to group III

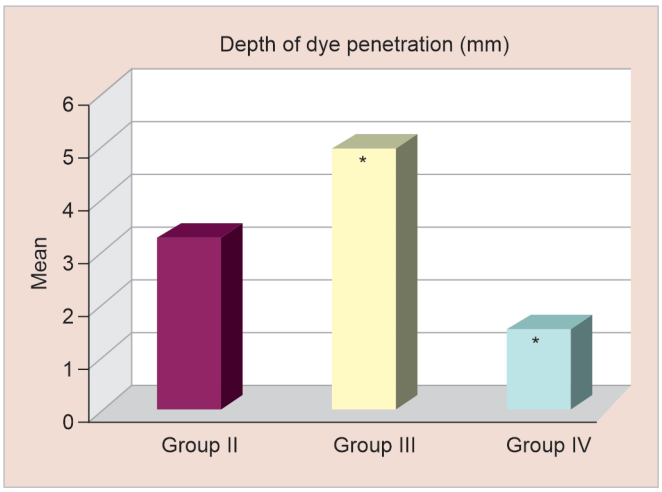

Fig. 4: Comparisons of difference in mean depth of dye penetration between three groups. *$p < 0.05$ as compared to group II

The solubility of sealers is another factor that decreases their efficiency. Thus, ideal sealers should have low solubility.[18] The smear layer is another reason for microleakage, and it also prevents the flow of sealers into canals.[19,20] Its removal is recommended with successive application of ethylenediaminetetraacetic acid (EDTA) and NaOCl solution.[13,21,22] Proper flow of sealers along dentinal walls and gutta-percha prevents leakage of fluids, thereby avoiding reinfection, and it should also have minimum solubility for proper flow.[23]

In the present study, all 16 teeth were sectioned at a consistent working length of 12 mm apical to CEJ as recommended by Sultana et al.[16]

Many methods are used to evaluate the microleakage of sealers. Some are dye penetration, electrical methods, fluid filtration techniques, radioisotope tracing, and marginal adaptation. The bond strength and microleakage tests are the main methods used to evaluate dye penetration.[14]

This study used the dye penetration technique because it is simple, special equipment is not needed, and it is fast.[14] Indian ink is used because it flows deeply by the side of canals, has low weight, and can be handled easily.

The stereomicroscope is used for dye penetration evaluation because it quickly enlarges submicron-level defects, and the final assessment can be done using photomicrographs.[12] Another reason for using a stereomicroscope is the immense field depth, better magnification, and higher resolution.

The diaphanization technique is used in this study because it better illustrates the dye and provides good transparency. This method offers a more accurate assessment compared to longitudinal and transverse cutting techniques.

The study's findings revealed that all tested materials displayed microleakage. Group I—Endoseal showed the maximum dye penetration, which could be attributed to the material's high solubility, which weakened the adhesion between the sealer and gutta-percha. Besides that, the longer setting time of the sealer also makes this sealer inferior to other sealers. The result of the present study was similar to a study done by Poggio et al.[19]

On intergroup comparison of sealers, results showed different and lower depth of dye penetration of group II (Apexit), group III (MTA Fillapex), and group IV (Ceraseal) compared to group I (Endoseal).

On the intergroup comparison, the best sealer was group IV (Ceraseal), followed by group II (Apexit), group III (MTA Fillapex), and the least effective was group I (ZOE).

Traditionally, sealers such as ZOE and $Ca(OH)_2$ have favorable properties but show more dye penetration because of weak bonding and poor dimensional stability.[3]

Lankar et al.[6] compared the sealing ability of ZOE-based sealer, $Ca(OH)_2$, resin sealer, and tricalcium phosphate-based sealer and showed that ZOE-based sealer has higher microleakage.

On intergroup analysis, as shown in Figure 3, $Ca(OH)_2$ exhibited less microleakage than ZOE because of volumetric expansion in $Ca(OH)_2$, which is not seen in ZOE-based sealers. Beneficial biological reactions of $Ca(OH)_2$ made it a suitable filling material.[6] The results were similar to the study by Tomer et al.[3] and Lankar et al.[6]

Further, when comparing MTA Fillapex with $Ca(OH)_2$, MTA Fillapex had more leakage than Apexit. The reason for increased microleakage with MTA Fillapex compared with $Ca(OH)_2$ could be that MTA Fillapex expands after 28 days.

MTA setting leads to the hydration of inorganic oxide compounds, resulting in the production of $Ca(OH)_2$ and calcium silicate hydration phases, which in turn lead to expansion at its margin, improving the seal and reducing its microleakage, whereas $Ca(OH)_2$ shows volumetric expansion that reduces microleakage, which increases its solubility. The results were consistent with the study conducted by Tomer et al.[3]

Thus, this study concluded that $Ca(OH)_2$ is superior to MTA Fillapex and ZOE (Endoseal) seal.

The smear layer is the leading cause of microleakage. Removing the smear layer from the apical third is challenging because it acts as a physical barrier, interfering with the adaptation of sealer to dentin.[24] This has led to various research efforts and the introduction of new sealers, such as bioceramic sealers.

In this study, the bioceramic sealer is compared with three sealers. The newly introduced bioceramic sealer used in this study is Ceraseal. It has been concluded that Ceraseal has better sealability than other sealers used in the study.

The reason behind the high sealing ability of Ceraseal and higher resistance to leakage is the formation of a mineral infiltration zone (hydroxyapatite crystals).[8] These sealers have calcium silicate and calcium phosphate monobasic.

Ceraseal is a sealer based on tricalcium silicate. It contains calcium silicate and calcium hydroxide, both of which release ions. This makes it a bioactive sealer. Its advantage is that it forms hydroxyapatite and is biocompatible. This biocompatibility is due to the presence of phosphate ions.[25,26]

Furthermore, in a comparative evaluation of group I with group IV, Ceraseal was considered the best. ZOE has high leakage when compared to Ceraseal because it has low tensile strength, poor adhesive properties, and solubility of sealers resulting in dissociation of zinc eugenolate into ZnO and $Zn(OH)_2$ ions[14,15] whereas in Ceraseal, hydroxyapatite bond formation increases its resistance to leakage, thereby increasing its sealing ability in comparison to other groups.

In another study by Ricardo et al.,[10] bioceramic sealer yielded promising results and showed higher flow of bioceramic sealers and marginal adaptation. The results were similar to our study.

Group IV (Ceraseal) demonstrated the highest sealing ability at the dentin interface compared to the other groups in this study. The superior performance of the bioceramic sealer can be attributed to its hydrophilicity, biocompatibility, nontoxic nature, and ability to form a hydroxyapatite bond.[27]

Within the study's limitations, the sealability of Ceraseal was found to be maximum. However, other root canal sealers used in the study showed microleakage. Therefore, proper disinfection and root canal sealing are crucial for achieving the best results.

Conclusion

The penetration depth was lower in groups II, III, and IV compared to group I. It was also lower in both group II and group IV compared to group III. Moreover, it was lower in group IV compared to group II.

On comparing the microleakage of four groups, all groups exhibited statistically significant penetration depth. The lowest microleakage was seen with group IV, followed by group II, group III, and group I, which was maximum (group IV < group II < group III < group I).

On analyzing the sealing ability for groups, that is, group I, group II, group III, and group IV, Ceraseal was a superior material when compared with Apexit, followed by MTA Fillapex and Endoseal.

Limitations of Study

This study utilized Indian ink as a dye penetration agent. Using alternative dye solutions (e.g., 0.2% rhodamine, 1%, or 50% silver nitrate) might have resulted in different outcomes.

The *in vitro* penetration of dye into canals should not be directly comparable with *in vivo* leakage of irritants in the canal.

The sample size was small, and it did not mimic the diverse conditions present in the oral cavity.

Future Scope of Study

This study was done under *in vitro* conditions. Long-term *in vivo* studies on large samples are required to check for the sealing ability of different root canal sealers before routine clinical usage.

The newly introduced bioceramic sealers yield promising results and have better sealing ability, though further investigations are needed for bioceramic seals.

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