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"A COMPARATIVE EVALUATION OF VARIOUS ENDODONTIC IRRIGANTS ON THE SEALING ABILITY OF BIODENTINE ON SIMULATED IMMATURE APEX- AN IN VITRO STUDY."

Paediatric Dentistry

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ABSTRACT

Background: The study aims to evaluate the usefulness of biodentine as a root end-filling material following the removal of smear layers, as well as the impact of biodentine's sealing ability when irrigated with normal saline, chlorhexidine, EDTA, and HEBP **Objective:** To evaluate and compare the sealing ability of biodentine after using various irrigating solutions on simulated immature apex. **Methods:** forty extracted human maxillary central incisors were collected for the study with 10 samples per group. The apices were removed by cutting with a diamond disc- 2mm from the apical root end and the teeth were decoronated in an attempt to standardize the working length of all specimens to 15 mm. A standardized open apex was created using Peeso drills #1 to #6 in a way that drill #6 was allowed to pass 1 mm beyond the apex. All samples were randomly divided into 4 groups: Group–I: normal saline; Group–II: 2%chorhexidine; Group III:17%EDTA; Group–IV: HEBP. After irrigation, 3 mm of apical plug was formed using biodentine in all the samples. Specimens were then immersed in 2% methylene blue for 48 hours after obturation with gutta-percha. The specimens were analyzed under a stereomicroscope at 20x magnification. The dye penetration was calculated using computer software. **Result:** The data was statistically analyzed using One Way ANOVA and Tukey's post hoc test. The mean of dye penetration varied across the irrigants, with highest mean value obtained in Normal saline (2.71) followed by CHX (2.24), EDTA (1.97), and HEBP (0.93). **Conclusion:** It was concluded that irrigation with HEBP significantly influenced the sealing ability of Biodentine followed by EDTA, chlorhexidine and normal saline Keywords: Biodentine, Chlorhexidine, EDTA, HEBP.

KEYWORDS

INTRODUCTION

One of the most important requirements for a successful endodontic treatment is a three dimensional, fluid-tight seal of a well cleaned root canal. Routine endodontic care is sufficient to accomplish this for a predictable and long-term prognosis of the teeth. But some clinical circumstances, such as different anatomical, technical, pathological, and iatrogenic consequences, challenge standard practice such that their prognosis can be predicted. One such situation is immature nonvital teeth. Large open apices, divergent root walls, thin dentinal walls that are prone to fracture, and frequent periapical lesions make teeth with inadequate rhizogenesis or blunderbuss canals particularly difficult to three-dimensionally seal.[1]

Root canal filling achieves three significant objectives: (i) Elimination of residual bacteria from the root canal system (ii) Prevent the entry of fluid from the periapical tissues (iii) Prevent coronal microleakage [2] Therefore, eliminating microorganisms and creating a fluid-tight seal are the most crucial elements of a successful endodontic procedure. Periapical pathosis may not always be resolved by the application of more effective instruments and materials, as well as by newer ideas and methods. In situations where endodontic therapy fails, surgery can be necessary.[3] A retrograde filling material is usually used to seal the root-end cavity. Preventing micro leakage, biocompatibility and material stability in the apical tissues are very important. The retrograde filling material should adhere to the walls of the cavity and resist resorption and moisture ingress. A good quality apical root canal filling is essential to endodontic surgery success.[4]

However, despite these developments, clinical shortcomings/failures continue to exist. Microleakage is the most frequent cause of endodontic failure, defined as the clinically undetectable passage of bacteria, fluids, molecules or ions between the tooth and the restorative or filling material. Methylene blue dye penetration is one of the most frequently employed methods for measuring microleakage. Since the dye molecules in the methylene blue solution are 103 times smaller than those in bacteria, 144.5% of the test specimens may demonstrate excessive dye penetration. The assessment of microleakage in Biodentine as apical barriers is necessary to overcome the seepage of bacterial load and maintain integrity.[5]

Biodentine is a water-based material containing tricalcium silicate. It has been introduced to dentistry as a "dentin replacement material" in 2009. It contains tricalcium silicate, dicalcium silicate, tricalcium aluminate and tetra-calcium aluminoferrite. Biodentine is produced in the form of a capsule with powder and liquid in ideal proportions. The powder content contains 80.1% tricalcium silicate, 14.9% calcium carbonate and 5% zirconium oxide. Calcium carbonate is for calcium filler content, improve biocompatibility and reduce setting time; zirconium oxide is used to increase radiopacity. Biodentine has been stated to have broader clinical use than the Mineral Trioxide Aggregate (MTA) including pulp capping, pulpotomy, perforation repair, bifurcation lesion repair, internal and external root resorption, apexification, liner material for the coronal restorations as well as deep cervical and root cavities. Biodentine has been shown to have a greater ability to seal, a higher compression strength, a faster setting time (12 minutes), less colour change, and better antimicrobial properties in studies.[6]

Root resection during periapical surgery creates a smear layer. Some investigations have focused on its removal while others have considered its effects on apical and coronal microleakage, bacterial penetration of the tubules, and the adaptation of root-end filling materials.[7] It has been reported that application of acids or chelating agents, can remove smear layer and improve the adhesion and penetration of root-end filling materials. This makes the root surface more biocompatible, optimizing periodontal healing without interfering with apical root end filling seal.[8] To eliminate the smear layer, several chemicals such as sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA) are utilized. The elimination of the smear layer in the apical third of the root canal system using 17% EDTA has been reported to be ineffective.[9] After preparation, chelating agents and acids are recommended for smear layer removal. Ethylenediaminetetraacetic acid (EDTA) is the most widely substance used due to its capacity to dissolve the inorganic portion of the dentine. Several substances are used during endodontic preparation. The most common irrigant used is sodium hypochlorite (NaOCl) due its ability to dissolve organic tissue and antimicrobial activity. In addition, chlorhexidine (CHX) has been proposed due to its reduced toxicity, antimicrobial activity and substantivity.[10] HEBP (1-hydroxyethylidene-1, 1-bisphosphonate [BP]), also known as etidronic acid or etidronate, has been proposed as a potential alternative to EDTA or citric acid because this agent shows no short-term reactivity with NaOCl.[11] The aim of the study to evaluate the usefulness of biodentine as a root end filling material following the removal of smear layers.

The purpose of the study was to evaluate how irrigation with normal saline, chlorhexidine, EDTA, HEBP influence the sealing ability of

biodentine when used as root end filling material.

MATERIALS AND METHOD

The study was carried out in the Department of Pediatric and Preventive Dentistry, Darshan Dental College and Hospital, Udaipur.

Source Of Data

Forty extracted human permanent maxillary central incisor teeth were collected from Department of oral and maxillofacial surgery, Darshan Dental College and Hospital, Udaipur.

Inclusion Criteria-

- Extracted permanent maxillary central incisors.
- Straight root.

Exclusion Criteria-

- Endodontically treated teeth
- Teeth with root caries
- Teeth with calcified canal
- Teeth with multiple canals
- Teeth with curved canal

Methodology:

Forty extracted intact permanent maxillary central incisors were collected from the department of oral and maxillofacial surgery darshan dental college and hospital, loyara (Udaipur). Preoperative radiographs (RVG- dental imaging system SOPRO ACTEON) was taken in mesiodistal and buccolingual directions to confirm the presence of a single unmanipulated root canal without root caries, root resorption, or calcification.

The teeth were cleaned using ultrasonic scaler to remove the soft tissue debries and calculus following which they were stored in 10% formalin. Coronal access cavity was prepared using a size 3 round bur and safe end bur using high-speed contra angle handpiece under water cooling.

The apices were removed by cutting with a diamond disc under water coolant - 2mm from the apical root end in an attempt to standardize the working length of all specimens to 15± 1 mm. Working length was determined by measuring a size 10 K-file, which was advanced into the canal until just visible at the apex and then subtracting 1 mm from this length.

The samples were instrumented up to Protaper #F5 under constant irrigation using 5ml side vented needle. A standardized open apex was created by means of Peeso drills #1 to #6 in a way that drill #6 was allowed to pass 1 mm beyond the apex.

All The Samples Were Randomly Divided Into 4 Groups:

Group 1- Irrigated with normal saline
Group 2 - Irrigated with chlorhexidine
Group 3 - Irrigated with EDTA
Group 4 - Irrigated with HEBP (Hydroxyethylidene bisphosphonate)
3 mm of apical plug was formed using biodentine in all the samples which was confirmed using radiograph followed by obturation of the canal by gutta percha using lateral condensation technique. Two layers of nail varnish was applied to all the samples except for apical 3 mm that was remained exposed to the dye solution. Specimens were then be immersed in 2% methylene blue for 48 hours.

After removal from the dye solution, the specimens were rinsed and dried. The roots were cut longitudinally following the principal axis of the root. The specimens were then analyzed under stereomicroscope for dye penetration in the apical third of the canal at 20x magnification.

Observation, Calculation And Statistical Analysis

Data was entered into Microsoft Excel spreadsheet and was checked for any discrepancies. Summarized data was presented using Tables and Graphs. The data was analysed by SPSS (21.0 version). Shapiro Wilk test was used to check which all variables were following normal distribution. Data was normally distributed therefore, inferential statistics were performed using the parametric test. For comparison of continuous quantitative data among more than two groups, ONE WAY ANOVA followed by Tukeys test were used. Post hoc pairwise comparison was done using Tukeys test. Level of statistical significance was set at p-value less than 0.05 (*).

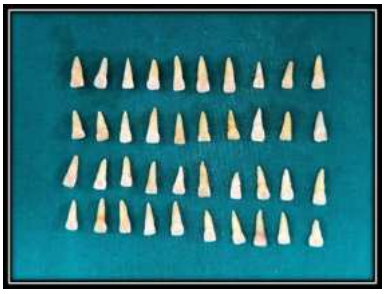


Figure 1: 40 Extracted human permanent central incisors

Figure 2: Sample divided in 4 groups after irrigation

Figure 3: The root cut longitudinally

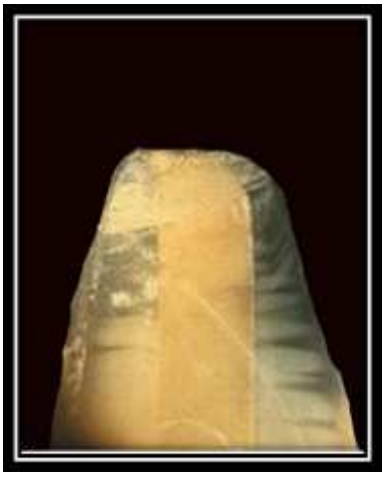


Figure 4: Group I sample viewed under stereomicroscope

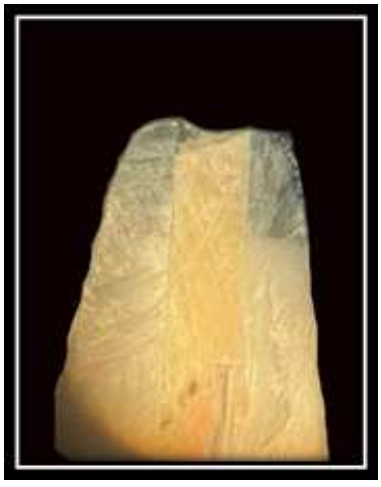


Figure 5: Group II sample viewed under stereomicroscope

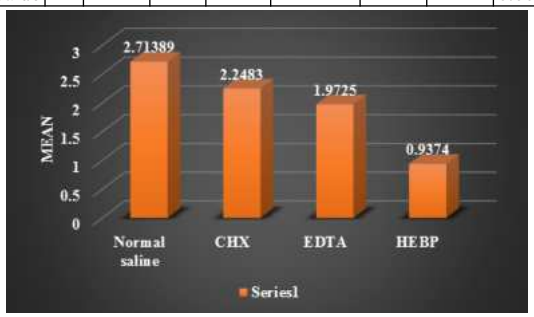
Figure 6: Group III sample viewed under stereomicroscope

Figure 7: Group IV sample viewed under stereomicroscope

RESULTS

Table I: An Intergroup Comparison Of Dye Penetration Of Endodontic Irrigants For The Sealing Ability Of Biodentine On Simulated Immature Apex.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Normal saline	10	2.71389	.136380	.045460	2.60906	2.81872	2.510	2.864
CHX	10	2.24830	.175399	.055546	2.12283	2.37377	2.093	2.659
EDTA	10	1.97250	.205745	.065062	1.82532	2.11968	1.781	2.280
HEBP	10	.93740	.097187	.030733	.86788	1.00692	.803	1.098
P value								0.001*

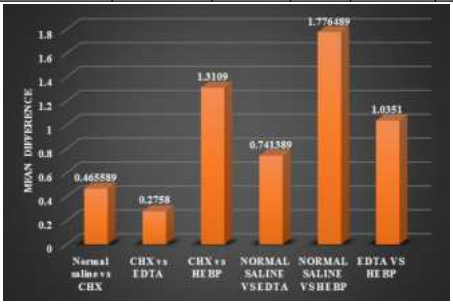


Graph I An Intergroup Comparison Of Dye Penetration Of Endodontic Irrigants For The Sealing Ability Of Biodentine On Simulated Immature Apex.

Table I And Graph I: This table presents an intergroup comparison of dye penetration for four different endodontic irrigants - Normal Saline, CHX, EDTA, and HEBP - on Biodentine placed in simulated immature apex models. The mean dye penetration values range from 2.71389 ± 0.13638 for Normal Saline (highest penetration) to 0.93740 ± 0.09719 for HEBP (lowest penetration). The p-value of 0.001 indicates a statistically significant difference among the groups. These results suggest that HEBP provides the best sealing ability (least dye penetration), whereas Normal Saline shows the greatest dye penetration, indicating a poorer seal under the conditions tested.

Table II: Post Hoc Comparison Of Endodontic Irrigants On The Sealing Ability Of Biodentine On Simulated Immature Apex

	Mean Difference	Std. Error	P value	95% Confidence Interval	
				Lower Bound	Upper Bound
Normal saline vs CHX	.465589*	.073337	.001*	.26781	.66337
CHX vs EDTA	.275800*	.071381	.002*	.08329	.46831
CHX vs HEBP	1.310900*	.071381	.001*	1.11839	1.50341
NORMAL SALINE VS EDTA	.741389*	.073337	.001*	.54361	.93917
NORMAL SALINE VS HEBP	1.776489*	.073337	.001*	1.57871	1.97427
EDTA VS HEBP	1.035100*	.071381	.001*	.84259	1.22761



Graph II Post hoc comparison of Endodontic Irrigants on the Sealing Ability of Biodentine on Simulated Immature Apex

Table II And Graph II: This table shows the post hoc pairwise comparisons between each irrigant. Every comparison yields a p-value < 0.05, indicating significant differences in dye penetration across all pairs. Specifically, Normal Saline has significantly higher dye penetration than CHX, EDTA, and HEBP. Similarly, CHX shows higher penetration compared to EDTA and HEBP, and EDTA penetrates significantly more than HEBP. Overall, HEBP exhibits the lowest dye penetration among the tested irrigants, while Normal Saline demonstrates the highest, underscoring a clear hierarchy in sealing ability when using Biodentine in a simulated immature apex.

DISCUSSION

The aim of endodontic therapy is cleaning, shaping, and obturating the root canal system in three dimensions. The lateral canal and auxiliary canal channels, located at the apical delta, serve as the channels for communication between the main body of the root canal and the periodontal ligament region. Microorganisms may find a home in these pathways. By sealing the root canal system with sufficient and effective root end filling material, the surgical endodontic treatment aims to stop bacteria and their byproducts from invading the periradicular tissues. Apical periodontitis is caused by these root canal irritants that leak into the periradicular tissues. When root canal therapy repeatedly fails, periradicular surgery may be necessary. To achieve a tight apical seal and prevent the microorganism and its byproducts from entering the periapical area, this involves exposing the apex, resecting the root, preparing the root end, and filling the root end.[12] Other than fluid tight seal, factors which determine the periapical healing are biochemical preparation, irrigant used, intracanal dressing, root canal filling, apical limit of the obturation and systemic factors[13]. It also it relies on the filling methods, the chemical and physical characteristics of the sealer, and whether a

smear layer is present[14]. The smear layer that forms during endodontic therapy is primarily made up of organic materials including blood cells, bacteria, and pulp tissue remnants as well as inorganic components like dentin debris[15]. While some studies have concentrated on its removal, others have examined its effects on bacterial tubule penetration, apical and coronal microleakage, and the adaptability of root-end filling materials[7].

As per our best knowledge, there has been no study investigating the use of HEBP, EDTA, chlorhexidine, and normal saline as an irrigant in root-end cavities and assessing the effect on the sealing ability of biodentine as a root-end filling material. Hence, the present study was designed to assess the apical seal obtained after root-end cavity irrigation with HEBP, EDTA, chlorhexidine, and normal saline with Biodentine as a root-end filling material.

In our study we created simulated immature apex by peeso reamer #1 to #6 in a way that drill #6 was allowed to pass 1 mm beyond the apex. According to study conducted by Medha Roy (2024), created a simulated immature apex for the evaluation of microleakage of mineral trioxide aggregate and biodentine as an apical barrier. To simulate young permanent teeth, samples were instrumented using a peeso reamer with a diameter of 1.7 mm. This method aimed to simulate an open apex similar to Cvek's stage 3 root development[5]. Vineeta Nikhil (2016) evaluated and compared the sealing ability of mineral trioxide aggregate (MTA) with three different methods. They created simulated immature apex using rotary ProTaper files till F4. For the simulation of teeth with immature apices, Peeso reamers between #1 and #6 were introduced in the root canals, and a #6 Peeso reamer was allowed to protrude 1 mm beyond the apex[16].

Biodentine new bioactive calcium silicate-based cement has been recently launched in the dental market as a 'dentin substitute'. This new biologically active material aids its penetration through opened dentinal tubules to crystallize interlocking with dentin and provide mechanical properties. Biodentine has been formulated using MTA based cement technology and hence; claims improvements of some of the properties such as physical qualities and handling, including its other wide range of applications like endodontic repair and pulp capping in restorative dentistry[17]

Apical preparation forms a 2–5 μm thick superficial smear layer packed into dentinal tubules up to 40 μm. This interferes with the adhesion of filling materials to dentin walls and compromises the disinfection of dentinal tubules by preventing the penetration of disinfecting solutions [9]. Study done by Chhaparwal S (2017) research has demonstrated that 7% maleic acid effectively removes the smear layer, particularly in the apical third of the root canal system, thereby improving the sealing properties of Biodentine[8]. Additionally, another study done by Thakkar R (2021) found that irrigating root-end cavities with Irritrol, a solution combining chlorhexidine and EDTA, enhances the apical seal of Biodentine by effectively removing smear layer, leading to reduced microleakage [18].

Biodentine causes alkaline corrosion (caustic etching) on the hard tissue, which leads to a mineral interaction zone. A diffusion of Biodentine up to 10-20 μm into the dentine tubule is observed. Thus, Biodentine tags can form within the dentin tubules, creating micromechanical anchoring that enhances Biodentine's adhesive properties [19]. Due to micromechanical retention of biodentine the removal of smear layer with irrigation is necessary to improve the sealing ability of biodentine.

The effect of various irrigation solutions on sealing ability of Biodentine, when used as root-end filling material, is currently lacking. Hence, the aim of this study was to evaluate the effect of 17% EDTA, HEBP, Chlorhexidine and normal saline irrigation on the apical sealing ability of Biodentine when used as a root-end filling material.

Chlorhexidine (CHX) is commonly used final irrigant in endodontics because of its antimicrobial properties and adhesion into root canal dentin. CHX also decreases the activity of collagenolytic enzymes, matrix metalloproteinases (MMPs) in radicular dentin. This effect could have a positive influence on the sealing ability and adhesion of the root canal filling material. CHX improves the long-term adhesion to dentin with composite fillings and it may also at least moderately improve the immediate and the long-term post adhesion to root dentin. CHX is said to improve the sealer wettability [20].

In our study, according to table 1 and Graph 1, the mean values obtained for group I (normal saline) was 2.71 and group II (CHX) was 2.24. These values were significantly less than normal saline which suggests lower apical microleakage compared to normal saline irrigation. The reason for this could be less penetration of CHX into the dentinal tubules of the apical dentin and lower efficacy to remove smear layer compared to normal saline.

Similar study by R.M. Lindblad (2020) evaluated the long-term effect of chlorhexidine (CHX) and dimethyl sulfoxide (DMSO) on the sealing ability of Biodentine and ProRoot MTA. The leakage increased significantly during the 6-month storage in all groups except in Biodentine CHX group and Biodentine-DMSO group. The results indicate that CHX does not have the same kind of effect on preserving the dentin-obturation inter-face as it has with adhesive resins to coronal dentin. The CHX inhibition of dentin endogenous enzymes may not be as important in root canals as it might be with adhesives [20].

Many irrigants have got smear layer removing ability, of which the commonly used irrigants is 17% EDTA. It forms calcium chelates by acting as a chelating agent for inorganic divalent cations, including calcium ions [9].

Using EDTA, a calcium-chelating agent and sodium hypochlorite (NaOCl) solution, an organic tissue solvent, in alternating ways to remove the smear layer51.The most common irrigant used is sodium hypochlorite (NaOCl) due its ability to dissolve organic tissue and antimicrobial activity. Ethylenediaminetetraacetic acid (EDTA) is the most widely substance used due to its capacity to dissolve the inorganic portion of the dentine[10].

EDTA reaction with calcium ions in dentine results in calcium chelation, promoting decalcification of dentine at approximate depths of 20–30 μm within 5 min[11]. EDTA recommended in combination with different concentrations of NaOCl for the complete elimination of the smear layer [21].

In our study, according to table I and Graph I, the mean value obtained for dye penetration for group 3 (EDTA) was 1.97 which is lesser than normal saline and chlorhexidine and higher than that obtained for HEBP irrigation (0.93). The possible reason could be EDTA effectiveness of removing the smear layer, which enhances the adaptation of Biodentine to the dentinal walls and reduces microleakage.

Similary, in an in-vitro study conducted by Sree Gowri (2022) to assessed the sealing ability of Biodentine (BD) and mineral trioxide aggregate (MTA) as retrograde filling materials after irrigation with 17% ethylenediaminetetraacetic acid (EDTA) and QMix irrigating solutions. In this study, normal saline had the highest microleakage, followed by 17% EDTA, and QMix had the lowest microleakage [9].

Another similiar study conducted by Shubha Chhaparwal (2017) evaluated the effect of 17% ethylenediaminetetraacetic acid (EDTA) and 7% maleic acid (MA) irrigation on microleakage of mineral trioxide aggregate (MTA) and Biodentine (BD) when used as a root-end filling material. They found that saline group demonstrated significant higher leakage than that of 17% EDTA and 7% MA in both MTA and BD groups. This could be attributed to the improper marginal adaptation of BD to root canal walls treated with 17% EDTA. It has been reported that the adhesion of BD to the root canal walls is most likely through the tag like structures formed within the dentinal tubules leading to micromechanical retention [8].

In contrast to our study, results were obtained by Ahmed K. Al-Zubaidi (2014) stated that normal saline and NaOCl increase the sealing ability of biodentine and observed that 17% EDTA for 10 min significantly increased dye penetration in teeth treated by Biodentine, compromising its sealing ability. This can be explained on the basis of calcium chloride present in the Biodentine liquid that's supplied by the manufacture. The addition of CaCL2 is intended to reduce the setting time and improve physiochemical properties by its ability to penetrate the pore of the cement, strongly accelerating the haydration of the silicate and leading to their faster crystallization [22]

HEBP (1-hydroxyethylidene-1, 1-bisphosphonate [BP]), also known

as etidronic acid or etidronate, has been proposed as a potential alternative to EDTA or citric acid because this agent shows no short-term reactivity with NaOCl[23].

Its compatibility with sodium hypochlorite (NaOCl) allows for simultaneous use without diminishing the antimicrobial efficacy of NaOCl. Studies have demonstrated that HEBP effectively removes the smear layer with less dentin erosion compared to EDTA. Additionally, HEBP has been shown to enhance the bonding quality of certain root canal sealers. Its mild chelating properties and biocompatibility make HEBP a promising alternative in root canal irrigation protocols.[24,25] In our study, according to table 1 and graph 1, the mean values obtained for group I (normal saline) was 2.71, group II (CHX) was 2.24, group III (EDTA) was 1.97 and group IV (HEBP) was 0.93. Hence, the results of our study suggest that HEBP irrigation has the highest sealing ability of biodentine, followed by EDTA, chlorhexidine and normal saline. The possible reason for HEBP increasing sealing ability of biodentine attributed to the possible low surface tension of the solution, increasing its diffusion into the dentinal tubules of apical third and efficacy to remove smear layer.

In accordance to our study, the in vitro study conducted by Sethuparvathi Anitha (2021) evaluated and compared the apical seal obtained with Biodentine after conditioning of root end with three different solutions HEBP, chitosan, and EDTA and concluded that irrigation with newer agents significantly influenced the sealing ability of biodentine. Root-end irrigation with 18% HEBP and 0.2% chitosan showed the least microleakage when compared to 17% EDTA. This could be attribute to HEBP as a chelating agent has optimal effects on Ca/P ratio, dentin surface roughness, and microhardness with no erosive effects on dentin wall as compared to the other agents.[11]

Similarly study by Tartari T et al (2018) have shown that HEBP caused the removal of phosphate, exposure of the collagen matrix, and an increase in the amide III/phosphate ratio, which was concentration dependent and concluded that HEBP is potent irrigant to remove smear layer effectively [26].

In a study by Tartari T et al in 2013, stated that when HEBP was compared with other widely used chelating agents like citric acid and EDTA, HEBP had lower values on root dentine microhardness than citric acid and EDTA. The cause can be linked to HEBP's inferior chelating ability compared to other chelating agents due to its interference with the chemical composition of the dentin surface [27].

Several methods have been employed to assess sealing ability of root end filling materials such as dye leakage, fluid filtration, bacterial penetration, radiolabelled isotopes, electromechanical tests, scanning electron microscopy and others [8,28].

It is important to emphasize that no standard method of microleakage assessments exists and there is a lack of technical standardization even when the same methodology is used. The lack of standardization is probably the main reason why there are so many different methods to study the same phenomenon [28]. Dye penetration methods are commonly used in the detection of apical leakage. Dyes like Eosin, Methylene blue, Black India Ink, Procion brilliant blue, etc are used [29].

In our study, 2% methylene blue dye was used for 48 hours because its molecular size is similar to bacterial by-products such as butyric acid which can leak out of infected root canals to irritate periapical tissues, also it is easy to use, pH manipulation and availability add to its advantages. It also has the potential to enter the obturated canals through complex anatomies of apical third of the root canal or space between dentin-sealer-core material interfaces. The particle size of this dye (0.1-2 μm) is comparable to the size range of a number of endodontic pathogens and appears to be advantageous in endodontic dye leakage studies [30].

Similarly in a study conducted by Sakshi M and Mithra N. H. (2015) also used methylene blue dye 2% for 48 hours to evaluate the sealing ability in the apical third. Longitudinal root resection was done. The depth of dye penetration was evaluated under the stereomicroscope to examine the extent of microleakage[3]. Therefore, in our study we have used the flow of 2% methylene blue dye for 48 hours to evaluate the sealing ability of the apical plug of biodentine. The penetration of dye was measured in mm by a stereomicroscope.

In the present study, samples were viewed under stereomicroscope at 20X magnification. The amount of dye penetration in mm was measured for each group by Image J software. It has been stated by A Fabio et al in 2015 that examination by stereomicroscopy is reliable method and based on the technological developments measurement in millimetres is more accurate by digital method (software) when compared to visual[31]. Another study conducted by Chen et al in 2013 states that the best images were obtained by stereomicroscope at 20X when assessing the microleakage. The reason could be that, detecting leakage under stereomicroscope is easy and accurate because, colours of the dye are clear and distinguishable [22].

In our study, according to table I and Graph I, the mean values obtained for group I (normal saline) was 2.71, group II (CHX) was 2.24, group III (EDTA) was 1.97 and group IV (HEBP) was 0.93. The values obtained for group I were significantly more than group II, group III and group IV which means apical microleakage was highest with normal saline irrigation compared to Chlorhexidiene, EDTA and HEBP respectively.

Limitations:
These studies do not fully replicate in vivo conditions, where factors like blood contamination, periapical pressure, and immune responses can influence material performance. Standardized sample preparation may not accurately reflect natural variations in root canal anatomy. Additionally, the long-term degradation and aging of Biodentine in an oral environment cannot be precisely assessed. The choice of storage media may also impact results, as it may not truly simulate clinical conditions.

Further in-vivo research is needed to establish it as a potential irrigant since the clinical environment could not be simulated in this study.

Other advance method like fluid filtration, bacterial penetration, radiolabelled isotopes, electromechanical tests, scanning electron microscope could be used to assess sealing ability of biodentine.

CONCLUSION

Based on the study's limitation and the results obtained from the study, within the circumstances of our in-vitro study, it can be concluded that

1. The use of irrigation agents improved the sealing ability of Biodentine as a root end material.
2. Irrigation with HEBP (1-hydroxyethylidene-1, 1-bisphosphonate) showed the highest sealing ability of Biodentine when used as root end filling material.
3. 17% EDTA showed the second highest sealing ability of Biodentine.
4. Chlorhexidine showed less sealing ability in comparison to 17% EDTA and HEBP. Normal saline showed least sealing ability of Biodentine among all the irrigants.

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