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Research Article

COMPARATIVE EVALUATION OF EFFECT OF 17% ETHYLENEDIAMINETETRAACETIC ACID AND CARBOXYMETHYL CHITOSAN ON SEALING ABILITY OF BIODENTINE AND ENDOSEQUENCE AS RETROGRADE FILLING MATERIAL : AN IN VITRO SCANNINGELECTRON MICROSCOPIC STUDY

Dr. Aradhana Choudhari, Dr. Sanjay Patil, Dr. Vandana Gade, Dr. Hemlatha Kulkarni, Dr. Amber Raut and Dr. PraktanGire

The Swargiya Dadasaheb Kalmegh Smruti Dental College and Hospital Wanadongri, Hingna

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ABSTRACT

Aim: The purpose of this study was to evaluate the sealing ability of Biodentine and Endo Sequence with 17% EDTA and carboxymethyl chitosan (CMC) as retrograde smear layer removing agents using scanning electron microscopy (SEM). **Materials and Methods:** Forty human single rooted teeth were taken. Crowns were decoronated and canals were obturated. Apically roots were resected and retrograde cavities were done. Based on the type of retrograde material placed and the type of smear layer removal agent used for retrograde cavities, they were divided into four groups (N = 10): Group I 17% EDTA with Biodentine, group II CMC with Biodentine, group III 17% EDTA with Endo Sequence, and Group IV CMC with Endosequence. All the samples were longitudinally sectioned, and the SEM analysis was done for marginal adaptation. **Statistical Analysis:** Kruskal-Wallis and Mann-Witney analysis tests. **Results:** SEM images showed the presence of less gaps in group IV, i.e., CMC with Endo Sequence when compared to other groups with statistically significant difference. **Conclusion:** Within the limited scope of this study, it was concluded that Endo Sequence as retrograde material showed better marginal sealing ability.

INTRODUCTION

The presence of endodontic microbiota and biofilms in inaccessible areas cause failure in the endodontic treatment 1. When nonsurgical root canal treatment fails to treat periradicular lesions of endodontic origin, surgical endodontic treatment is preferred 2.

Biodentine is a relatively new material introduced as a dentine substitute. Biodentine powder is mainly composed of highly pure tricalcium silicate, which regulates the setting reaction. Other components are calcium carbonate (filler) and zirconium dioxide (radiopacifier). The liquid contains calcium chloride (setting accelerator), water reducing agent (super-plasticizer) and water. The super-plasticizer reduces the viscosity of the cement and improves handling 3. The manufacturer claims that this material can be used for pulp capping, pulpotomy, apexification, root perforation, internal and external resorption and also as a root end filling material in periapical surgery. In the previous studies, Biodentine showed biocompatibility and the ability to induce odontoblast differentiation and mineralization in cultured pulp cells 4. The main benefits of Biodentine over other calcium silicate based materials are the reduced setting time, better handling and mechanical properties 5. The importance of marginal adaptation is that it may have an indirect correlation with the sealing ability of retro- filling materials6.

Corresponding author:* **Dr. Aradhana Choudhari
The Swargiya Dadasaheb Kalmegh Smruti Dental College and Hospital Wanadongri, Hingna

Endo Sequence root repair material (ERRM) is a bioceramic material which is made out of calcium silicates, zirconium oxide, tantalum oxide, monobasic calcium phosphate, and fillers. The bioceramic material enters dentinal tubules and collaborates with the humidity available in the dentin because it is produced with nanosphere particles;6 this makes a mechanical forms during hardening of cement. Previous studies have proved that ERRM is biocompatible,6,7 seals root-end cavities,8 bioactive,9 antibacterial,10 and has high compressive strength.11 It sets in 20 min, and its hardening starts within the sight ofhumidity.7 The importance of marginal adaptation is that it may have an indirect correlation with the sealing ability of retro-filling materials 12.During retrograde cavity preparation, the smear layer is formed over the cut dentinal surfaces. The smear layer contains organic, inorganic material, bacteria, and theirby products13. It acts as a barrier between filling materials and the canal wall, thus compromising the formation of a satisfactory seal that may further lead to microleakage. 14

Mc Comb and Smith 15 were the first to describe the smear layer on instrumented root canal walls. Smear layer is defined as ‘an amorphous granular layer that consists chiefly of hydroxyapatite and altered collagen along with ground dentin, predentin, inorganic debris and organic components, such as pulp tissue remnants, odontoblastic processes, saliva, blood cells and bacteria'. No smear layer is found on areas that are not instrumented 16. According to many authors, this layer is directly created by instruments contacting the walls during canal preparation17,18.

Its removal is controversial. Some authors support the removal of the smear layer whileothers pursue the preservation of the smear layer. Authors support the removal of smear layer because some amount of bacteria may be lodged deep within the tubules and the smear layer may potentially be blocking and act as a barrier against the action of disinfecting irrigants during endodontic cleaning and shaping 19,20. It may also act as a substrate for bacteria, allowing their deeper penetration in the dentinal tubules 20.In order to dissolve debris and smear layer, chemical irrigation solutions are recommended along with mechanical instrumentation. One of the most common irrigant is sodium hypochlorite used mainly due to its tissue dissolving ability and antimicrobial property.

Although sodium hypochlorite appears to be the most desirable single endodontic irrigant, it cannot dissolve inorganic dentin particles and thus prevent the formation of a smear layer during instrumentation 21. In addition, calcifications hindering mechanical preparation are frequently encountered in the canal system. Demineralizing agents such as ethylenediamine tetraacetic acid (EDTA) 22 have therefore been recommended as adjuvants in root canal therapy.

The preparation of root canal using the most widely used irrigant for smear layer removal is ethylenediaminetetraacetic acid (EDTA) which is achieved by acting on an inorganic material 23,24,25.

Various agents, such as ethylenediaminetetraacetic acid (EDTA), mixture of tetracycline acid detergent (MTAD), organic acids have been introduced for smear layer removal, regarding orthograde and retrograde root preparation 26 . The alternating use of EDTA and sodium hypochlorite has been recommended for the efficient removal of the smear layer 27. However, the use of these solutions may cause periapical inflammatory reactions and reduce periapical healing. To minimize their harmful effects on periapical tissues the use of biocompatible solutions is essential.

Chitosan is a natural, glucosamine, and n‑acetylglucosamine's cationic amino polysaccharide copolymer obtained by the alkaline and the partial deacetylation of chitin which is obtained from shells of crustaceans and shrimps 28. It is the most abundant substance in nature after cellulose; making its use more eco‑friendly 29. It is biocompatible, biodegradable, nontoxic, having chelating property but limited solubility 30 . Chitosan possesses the high chelating capacity for various metal ions, including Zinc, Cobalt, Iron, Magnesium, and Copper ions (Zn2 þ, Co2 þ, Fe2 þ, Mg2 þ, and Cu2 þ, respectively) in acid conditions 31. Due to these properties, chitosan was used in various dental treatments such as in cases of direct pulp capping, in the treatment of dentinal tubule infection, 32 and in tissue regeneration in pulp wounds 33. Carboxymethyl Chitosan (CMC) has been introduced to overcome limited solubility of chitosan that is formed by Carboxymethylation of Chitosan 34 .

The treatment outcome is negatively affected by the failure of materials in achieving marginal adaptation and also due to the occurrence of cracks and spaces in the interface between the material and the dentin walls1.

Although there are inherent limitations in vitro studies conducted in laboratories, scanning electron microscopy (SEM) has been used in this study to evaluate the marginal adaptation of root-end filling materials 2.

Literature search revealed that, there have been few studies published which showed that the effect of 17% ethylenediaminetetraacetic acid and Carboxymethyl chitosan on sealing ability of Biodentine and Endosequence as retrograde filling material

So, the purpose of this study is to compare the effect of marginal adaptation of Biodentine and Endo Sequence, using 17% EDTA and CMC as retro smear layer removing agents.

The null hypothesis of the present study was that, there is no difference in The effect of EDTA and carboxymethyl Chitosan as retrograde smear layer removing agent, on the sealing ability of EndoSequence and Biodentine as root end filling material.

AIM

The aim of this study was to evaluate and compare the effect of 17% Ethylenediaminetetraacetic acid and 0.2 % Carboxymethyl Chitosan on sealing ability of Biodentine and Endosequence as retrograde filling material: an in vitro scanning electron microscopic study.

MATERIAL AND METHODS

1. The teeth were stored in 2% formalin before use. The crowns were decoronated to achieve a standardized working length of 16 mm from the root apex.
2. During instrumentation, the root canals were irrigated with 3 ml of 3% sodium hypochlorite and saline at each changes of file and dried with paper points.
3. The root canals were obturated with standard 2% gutta purcha coated with AH plus sealer using conventional lateral compaction technique. Excess gutta-percha was removed with a heat-carrier and remaining gutta-percha was vertically condensed at the canal orifices with a hand plugger.
4. Then the apical 3 mm of root were sectioned perpendicular to the long axis of the tooth with diamond disc.

Final Root-End Filling

1. Root end cavities were prepared with depth of 3 mm and width of 2 mm using no. 2 round Carbide bur.
2. All the teeth were divided into 4 experimental groups

Group1 17% EDTA used as smear layer removing agent and cavity filled with Biodentine (this material was prepared by adding 5 drops of liquid to the powder present in capsule).

Group2 0.2% carboxymethyl chitosan used as smear layer removing agent and cavity filled with Biodentine

Group3 17% EDTA used as smear layer removing agent and cavity filled with EndoSequence root repair material which is available in syringe form.

Group4 0.2% CMC used as smear layer removing agent and cavity filled with EndoSequence root repair material.

Root Sectioning and Scanning Electron Microscope

1. Materials were allowed to set and later all samples were sectioned longitudinally into two halves with the help of diamond disc for SEM evaluation.
2. The distance between the root-end filling materials and dentinal walls were measured under scanning electron microscope in µm.

RESULTS

Statistical analysis was done by using descriptive and inferential statistics using student's unpaired t test, one way ANOVA and Multiple comparison: Tukey Test and software used in the analysis was SPSS 24.0 version and p<0.05 is considered as level of significance.

Table 1 Comparison of biodentine as root end filling material in two groups Student's unpaired t test

Group	N	Mean	Std. Deviation	Std. Error Mean	t-value
17% EDTA	10	2.15	0.49	0.15	2.39
Carboxymethyl Chitosan	10	1.72	0.28	0.08	p=0.028,S

Table 2 Comparison of Endosequence as root end filling material in two groups Student's unpaired t test

Group	N	Mean	Std. Deviation	Std. Error Mean	t-value
17% EDTA	10	1.68	0.23	0.07	2.51
Carboxymethyl Chitosan	10	1.34	0.38	0.12	p=0.022,S

Table 3 Comparison between four groups Descriptive Statistics

Group	N	Mean	Std. Deviation	Std. Error	95%Confidence Interval forMean Lower Bound	Upper Bound	Minimum	Maximum
Biodentine with 17% EDTA	10	2.15	0.49	0.15	1.79	2.50	1.70	2.90
Biodentine	10	1.72	0.28	0.08	1.51	1.92	1.40	2.30
Endosequence	10	1.68	0.23	0.07	1.51	1.84	1.10	1.90
Endosequence	10	1.34	0.38	0.12	1.06	1.61	.80	1.90

Table 4 One way ANOVA

Source of variation	Sum of Squares	df	Mean Square	F	p-value
Between Groups	3.309	3	1.103		
Within Groups	4.741	36	0.132	8.375	0.0001,S
Total	8.050	39			

DISCUSSION

Marginal adaptation is one of the desirable properties for a retrograde filling material. Scanning Electron Microscope (SEM) aids in assessing the marginal adaptation of the filling material to tooth interface under higher magnification 29. In many studies, dye penetration method was used for the assessment of microleakage; however, there are certain limitations for traditional dye leakage methods such as dissolution during the process, and it is also difficult to observe the maximum dye penetration depth 29. So in the present study, scanning electron microscope examination was used to determine the marginal adaptation of root-end filling materials. SEM examination is a suitable method for the assessment of marginal adaptation because of its high magnification and good resolution 1.

The study utilized 2% formalin for preservation of tooth specimen before use as it provides storage and sterilization of bovine teeth that are to be used in dental bonding studies in vitro. Sterile water was used for storage of extracted specimens until use to prevent dehydration which might adversely affect the properties of tooth structure and lead to bias in the outcome of the experiment 30.

Decoronation was done to obtain a root length of 16 mm with a diamond disk under sufficient water cooling. This might have potential damage in the root, but in general the development of cracks caused by the sectioning procedure seems to be unlikely. However it has been argued that according to the several studies the occurrence of cracks caused by the sawing procedure were rarely reported in the literature as long as sufficient cooling was applied 31.

Then patency was achieved and working length was established 1mm short of apex for the uniformity during the preparation as well as evaluation. It is also has advised controlling the length of samples, the canal diameter, and the canal anatomy to reduce the variability of these studies 32 . So, in this study to achieve standardized root length of 14mm, the crowns were removed at the cementoenamel junction using a diamond disc to eliminate any variables in access preparation, which is in agreement with Limkangwalmonkol S *et al* (1992)33

Apical ramifications and laterals canals are very common near root tip 34. Resection at the depth of 3 mm reduces the apical ramifications by 98% and lateral canals by 93% 34.

During preparations of root-end cavity, good visualization and easy access are the main criteria for choosing 0°, 30° or 45° resection angles 35. However, angled root-end resection also opens dentin tubules which can increase the risk of bacterial contamination and microleakage resulting in failure of endodontic surgery 35. Gagliani *et al*, and Gilheany*et al*. in their studies stated that microleakage increased significantly with increased angulations of the resected root-end 34,36.

Perpendicular resection minimizes the number of exposed dentinal tubules 37. Hence 3mm of root-end was resected perpendicular to the long axis of the tooth in this study.

In the present study root end cavity were prepared with the depth of 3mm and width of 2 mm using no. 2 round carbide bur. During retrograde cavity preparation, the smear layer is formed over the cut dentinal surfaces. The smear layer contains organic, inorganic material, bacteria, and their by-products 17 . It acts as a barrier between filling materials and the canal wall, thus compromising the formation of a satisfactory seal that may further lead to microleakage 18.

The presence of smear layer may inhibit or significantly delay the penetration of irrigating solutions, sealers, and medicaments into the dentinal tubules 17. There is a controversy regarding the presence and removal of smear layer. It is now generally advocated that the smear layer should be removed prior to the root canal obturation to facilitate better adaptation of the filling material to the root canal wall and to improve adhesion 42 .

In the present study EDTA was used as a retro smear layer removing agent as it is a gold standard chelating agent. studies have shown that 17% EDTA efficiently removes the smear layer from root canal walls 38,39,40 . Alternate use of EDTA and NaOCL as retro smear layer removing agents may cause periapical inflammatory reactions at surgical site 41 .

Calt *et al*. observed that usage of EDTA for prolonged periods caused excessive tubular and intertubular dentin erosion 42 .
So the use of biocompatible retro smear layer removing agents is essential. Chitosan and Carboxymethyl chitosan are more biocompatible and used as retro smear layer removing agents. Chitosan is natural polysaccharide obtained by the deacetylation of chitin. Chitin and chitosan do not cause any biological hazard and are inexpensive43.

Chitosan exhibits many biological actions such as antimicrobial, wound healing, mucoadhesive, sustained drug releasing property, chelating agent, and also as irrigating solution 44. However, one of the drawback of Chitosan is limited solubility.

CMC has been introduced to overcome the limited solubility of chitosan. It is obtained by carboxymethylation of chitosan. It is having good solubility when compared to chitosan 50 .As CMC is completely soluble in distilled water so the availability of chitosan is more. Hence, the chelating action is more pronounced when CMC is used.

In the present study after removal of smear layer retrograde filling were done by using Biodentine and Endo Sequence.

Biodentine is a new calcium alumino silicate cement has been developed by Septodont in 2009 with excellent biocompatibility, increased physico-chemical properties like short setting time and high mechanical strength, which makes it clinically easy to handle for endodontic cases and dentin restorative procedures.

The adhesion of Biodentine cement to dentin may result from the physical process of crystal growth within the dentinal tubules leading to micromechanical bonding 44

In the current study another root end filling material used was Endo Sequence. It is a bioceramic material composed of calcium silicates, zirconium oxide, tantalum oxide, calcium phosphate monobasic thickening agents, and proprietary fillers 45 . Endo Sequence has been manufactured to overcome some of the difficult handling characteristics of Mineral Trioxide Aggregate.

The result of this study suggests that Mean value of marginal gap for Biodentine level in 17% EDTA (group I) was 2.15±0.49 and in Carboxymethyl chitosan (group II) it was 1.72±0.28. By using student's unpaired t test statistically significant difference was found in mean value of marginal gap for Biodentine level between two groups(t=2.39,p-value=0.028).

Thus in the present study statistical analysis revealed that Biodentine exhibited superior sealing ability, when root surface were treated with 0.2% CMC rather than 17% EDTA.

Mean value of marginal gap for Endo Sequence level in 17% EDTA (group III) was 1.70±0.23 and in Carboxymethyl chitosan (group IV) it was 1.34±0.38. By using student's unpaired t test statistically significant difference was found in mean value of marginal gap for Endo Sequence level between two groups (t=2.51,p-value=0.022).

Thus in the present study statistical analysis revealed that Endo Sequence exhibited superior sealing ability, when root surface were treated with 0.2% CMC rather than 17% EDTA.

As per the observations made in samples of this study, chitosan worked better at the apical third than 17% EDTA with statistically significant difference. When chitosan was used as retro smear layer removing agent, the dentinal tubules were seen open but not widely enlarged and also there was no action on tubular and inter tubular dentin which is in accordance with the study conducted by Saghiri*et al*. 44 . This suggests that the action of 0.2% chitosan is softer on dentin than 17% EDTA. Studies have suggested that 17% EDTA erodes tubular and intertubulardentin.

A neutral EDTA solution has the ability to reduce the noncollagenous proteins (NCPs) component and mineral of dentin as described in recent studies. Because the content of NCPs is less in the apical third, the degree of chelation of EDTA is low in this part 44 this might be the reason for lower chelating action of 17% EDTA than 0.2% chitosan in thisstudy.

The better chelating agent was 0.2% chitosan when compared to apple cider vinegar, and 15% ethylenediaminetetraacetic acid (EDTA). This result was also in accordance with the study done by Mittal A *et al* which concluded that application of 0.2% of chitosan solution for 3– 5 min as the most viable combination for use on the root dentin 46 .

This result is in accordance with the study done by Aradhana Babu Kamble *et al* 47 which concluded that chitosan also carries the property of chelation and thus can be said a calcium depleting endodontic irrigant. Also they concluded that chitosan with minimal chelation produced cleaner dentinal walls with minimal erosion of intraradicular dentin and so removed smear layer efficiently.

Our result are in accordance with the study conducted by Aradhana babu kambe *et al* 48 where they concluded that a moderate concentration of 0.2% chitosan removes the smear layer with greater efficiency than 17% EDTA.

According to results of this study, when sealing ability of Endo Sequence and Biodentine as retrograde filling material is compared sealing ability of Endo Sequence was found to be statistically significant higher as compared to Biodentine irrespective of smear layer removing agent used. The reason may be particle size, premixed material that allows the better penetration of this material into the dentinal tubules and also bond to the adjacent dentin 61 and Biodentine is a separate powder and liquid 49 another difference for better sealing ability of this material may be it is directly applied over the prepared cavity and the by-products formed in the setting reaction of are hydroxyapatite and water. According to the manufacturers of Endo Sequence, water formed in this reaction is important in controlling hydration rate and setting reaction of this material 49. Furthermore, the thickening and filler agents added to ERRM to make its putty form might be associated with higher bond strength. The presence of zirconium oxide in the ERRM might also result in higher bond strength of ERRM 50.

Our results are in accordance with the study conducted by Bolla Nagesh *et al*. 39 which evaluated the sealing ability of mineral trioxide aggregate (MTA) and Endo Sequence with chitosan and Carboxymethyl chitosan (CMC) as retrograde smear layer removing agents using scanning electron microscopy (SEM) and concluded that Endo Sequence as retrograde material showed better marginal sealing ability.

CONCLUSION

1. So, within the limited scope of this study, it is concluded that CMC grops are an effective chelating agent with less chemical andphysical changes in radicular dentine and can be considered as a less invasive alternative or replacement to 17% EDTA groups.
2. Endosequence showed less marginal gap with statistically significant difference when compared with groups filled with biodentine as root-end filling material.

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