Comparative evaluation of the sealing ability of filling materials on root end cavities treated with smear layer removing agents: A confocal laser scanning microscopic study

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Abstract

Aim: This study aims to evaluate and compare the sealing ability of Biodentine™ and mineral trioxide aggregate (MTA) plus® on root end cavities treated with 17% ethylene diamine tetraacetic acid (EDTA), 0.2% Chitosan and 1% Phytic acid using Confocal Laser Scanning Microscope (CLSM)-An in vitro study.

Materials and Methods: Sixty extracted single rooted teeth were instrumented and obturated with gutta-percha. The apical 3 mm of each tooth was resected and 3 mm root-end preparation was made using ultrasonic tip. 17% EDTA (n = 20), 0.2% Chitosan (n = 20) and 1% Phytic acid (n = 20) was used as a smear layer removing agent and each above group was further subdivided and restored with a root end filling material, Biodentine (n = 10) and MTA Plus (n = 10). The samples were coated with varnish except at the root end and after drying, they were immersed in Rhodamine B dye for 24 h. The teeth were then rinsed, sectioned longitudinally, and observed under CLSM.

Results: In the present study, MTA Plus® treated with 1% Phytic acid showed the least microleakage followed by Biodentine™ treated with 1% Phytic acid which was statistically not significant. MTA Plus® treated with 17% EDTA showed the highest microleakage when compared to other tested groups. There was a significant difference in microleakage between MTA Plus® and Biodentine™ when treated with 17% EDTA and 0.2% Chitosan. However, more microleakage was seen with Biodentine™ group than MTA plus® group.

Conclusion: Root end cavities restored with MTA plus and treated with Phyitc acid showed superior sealing ability. Furthermore, smear layer removing agents will aid in better adaptability of root end filling material.

Keywords: Biodentine™; confocal laser scanning microscope; mineral trioxide aggregate Plus®; sealing ability; smear layer removing agents

INTRODUCTION

Thorough debridement of the root canal system and filling it with a three-dimensional obturation is the major goal of root canal treatment.[1] Persistence of periapical pathology, overfilled canals, ledges, canal obstructions, separated instruments, apical transportations, and perforations are the situations indicated for surgical approach. Endodontic surgery is indicated when nonsurgical endodontic treatment is unsuccessful. It involves exposure of the apex, apical root end resection, retrograde preparation, and inserting the retrograde filling material.[1] Retrograde filing material should have beneficial properties such as biocompatibility,

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good strength, optimum sealing ability, promote healing, radiopacity, easy manipulation and should not get affected by the presence of moisture. These are the prerequisites for the success of surgical endodontics.\(^\text{[2]}\)

Root end filling is the procedure in which apical root canal is filled through a root end cavity by an inert nontoxic material.\(^\text{[3,4]}\) An ideal retrograde filling material should adapt to the root canal walls to obtain a tight seal in root canal system, it should be radio-opaque, dimensionally stable, nontoxic, well tolerated by the periradicular tissues, induce healing. It should be easily manipulated, nonabsorbable and be unaffected by presence of moisture.\(^\text{[5]}\) Various retrograde filling materials have been routinely utilized as root end cavity preparation out of which mineral trioxide aggregate (MTA) Plus™ (Prevest Denpro, Loma Linda, California USA in 1993) and Biodentine™ (Septodont, India) have been preferred. MTA Plus has the similar physical and chemical properties as that of MTA Angelus® (Loma Linda University, California, USA 1993) but it sets faster than MTA. MTA is a calcium silicate-based cement and was developed by Torabinejad \textit{et al.}, in 1993. They recommended MTA for retrograde filling as it had better chemical and physical properties,\(^\text{[6]}\) superior biological compatibility with excellent sealing properties and regeneration of hard tissue.\(^\text{[6]}\) It has the property to differentiate in various types of cells by osteogenic and odontogenic differentiation. However, prolonged setting time, difficult handling, discoloration of tooth structure, and being expensive were some of the drawbacks of MTA.\(^\text{[6]}\)

Another popular root end filling material is Biodentine which is a new bioactive material similar to physical and biological properties to MTA.\(^\text{[7]}\) It contains tricalcium silicate, zirconium oxide and calcium carbonate, while the liquid is hydrosoluble polymer and calcium chloride as a setting accelerator.\(^\text{[7]}\) The material has been proposed as an alternative to MTA due to its shorter setting time, no tooth discoloration and easy handling properties.\(^\text{[8]}\) Reduction in the particle size, addition of calcium chloride accelerator to the liquid component and reducing the liquid content results in fast setting times of 9–12 min.

Root end cavity preparation with ultrasonic retrotip has various advantages over other methods like conventional burs because ultrasonic retrotip root end preparation reduces the dentinal tubules exposure at the resected root surface leading to reduced apical leakage by creating a conservative, smooth, deep, and more centrally placed cavity.\(^\text{[9]}\)

The elimination of smear layer during preparation of the root canal helps to achieve a three dimensional seal.\(^\text{[10]}\) Smear layer removal agents which are used routinely, like ethylene diamine tetraacetic acid (EDTA) have been used in this study.

EDTA has been an irrigant of choice among many clinicians for the removal of smear layer.\(^\text{[10]}\) EDTA, having antimicrobial activity and cleansing ability, causes excessive tubular and intertubular dentin erosion when used for prolonged periods.\(^\text{[10]}\)

Various other smear layer removing agents such as Chitosan and Phytic acid have been used. Chitosan is a natural polysaccharide and is biocompatible, biodegradable, and nontoxic. It has chelating properties but it limits solubility.\(^\text{[10]}\) Phytic acid is a saturated cyclic acid, which is less cytotoxic and biocompatible when compared to EDTA. It has the ability to eliminate the smear layer from prepared root canal walls.\(^\text{[10]}\)

Confocal Laser Scanning Microscope (CLSM) is used to evaluate the microleakage because it has the advantage of providing detailed information and creating sharp images of the interface. It is thus used to analyze the conjugation between dentin and retrograde filling material and to evaluate marginal adaptation.\(^\text{[11]}\)
The objective of this study was to evaluate the sealing ability of root end filling materials with smear layer removing agents.

MATERIALS AND METHODS

Sixty freshly extracted human permanent single rooted teeth were selected for the study. The teeth were verified radiographically as having single straight patent canals with fully formed apices, no cracks, no calcification, no internal resorption, or previous root canal treatment.

To standardize samples, teeth were decoronated to the length of 16 mm. Access preparation was done and cleaning and shaping was performed using step-back technique up to size 60K file, in an effort to standardize volume. Canals were irrigated with 3 mL of 3% NaOCl and saline was used as a final rinse.

The canals were coated with AH plus sealer and obturated with 2% gutta-percha points using conventional lateral compaction technique. Access cavity was sealed with composite resin restoration. The apical 3 mm of roots were sectioned perpendicular to the long axis of the tooth using an aluminum sectioning disc (Shofu) with water coolant. The apical cavity of 3 mm was prepared using an ultrasonic retropreparation diamond tip ((SYBRON ENDO; BK3-R)) with water coolant.

The apical cavity was then observed for the extent of dye penetration under CLSM. Rhodamine B dye gave a red-orange fluorescence when excited with green light of 546 nm wavelength. The amount of dye penetration was measured in μm using the ZEISS LSM IMAGE BROWSER SOFTWARE (Version 4.2.0.121) under 10X magnification.

RESULTS

The mean microleakage value was lowest in Group III (b) (1% Phytic acid + MTA Plus) when compared with other groups [Table 1]. The mean microleakage was highest in Group I (b) (17% EDTA + MTA Plus) when compared with the other groups [Table 1]. On Intergroup comparison between Group III (a) and Group III (b) the mean values obtained for Group III (a) (Biodentine) were higher than Group III (b) (MTA Plus) which was not statistically significant with $P = 0.814$.

On comparison between Group I and Group II, the mean value obtained for Group I (17% EDTA) was higher than Group II (0.2% Chitosan). On intergroup comparison between Group II (a) (Biodentine) and Group II (b) (MTA Plus), the mean value obtained for Group II (b) (MTA Plus) was lower than Group II (a) (Biodentine). Similarly in Group I, Group I (a) (Biodentine) had lower mean values than Group I (b) (MTA Plus). Both groups were statistically significant compared to other groups.
with $P = 0.001$. The dye penetration for Biodentine was highest in 0.2% Chitosan, followed by 17% EDTA and the least with 1% Phytic acid [Figure 1]. Whereas, for MTA Plus, the highest dye penetration was seen in 17% EDTA group, followed by 0.2% Chitosan and the least with 1% Phytic acid.

**DISCUSSION**

Inability to achieve an optimal apical seal due to morphological conditions and roots which have been retreated necessitates a periapical surgery. In such cases, a cavity preparation at the root end, thorough eradication of pathological periradicular tissue and a good retrofilling leads to a successful prognosis.

The degree of microleakage is also affected by the plane of sectioning; in this root end section angle was selected to be 90° as it has proved to be the most accepted in earlier studies.[12] Root end resection at 30° or 45° angle can have compromised healing because of open dentinal tubules, loss of dentin, cementum, and bone, more mechanical stress and errors in postoperative radiographs.[12] As lateral canals and apical ramifications are common in apical 3 mm so root resection at 3 mm depth was preferred which reduced apical ramifications by 98% and lateral canals by 93%.[12]

In the present study root end cavity preparation was done using ultrasonics because the major disadvantages of rotary-type bur retropreparations can be overcomed with miniaturized ultrasonic tips. With the help of a small size ultrasonic tip precise, class I cavity can be prepared down the long axis of the root end with bucco-lingual extension through the isthmus with minimal destruction of the canal morphology.[12] It has been proven by the previous studies that cavities prepared by small size ultrasonic tips are more precise and conservative.[13]

Apical microleakage is the most common cause responsible for the failure of endodontic therapy. Thus, it is important to select an ideal retrograde filling material that adapts to the dentinal walls which can be achieved by removal of the smear layer using suitable agents.[13] In the present study, after preparing class I cavities with ultrasonic tips, the root end cavities were treated with 17% EDTA, 0.2% Chitosan and 1% Phytic acid to remove the smear layer.

Various techniques have been used for detection and evaluation of microleakage around the retrograde filling material in which use of dyes as tracers is one of the oldest and most common method as it is inexpensive, nontoxic and leakage can be easily detected.[13]

In the present study, specimens were immersed in a 0.2% aqueous Rhodamine B dye for 24 h. Rhodamine B, is a water-soluble fluorescent dye which is easily detectable in a low concentration. It has low toxicity, is nondestructive, is stable in an aqueous environment and also in varying pH. It moves freely along the interface. Later, hemisection of the specimens was done longitudinally through mid-line of the retrograde filling material.[12]

In this study, CLSM is used to detect sealing ability of root end material over scanning electron microscope because it is a nondestructive technique of visualizing the extent of dye penetration. It has certain advantages in visualizing subsurface tissue features including clear indication of leakage limits, due to a lens focus which occurs some microns beneath the observed surface.[12] It reduces polishing artifacts that can increase dye penetration depth, eliminates stain spread caused by specimen sectioning, also eliminates the scattered, reflected, and fluorescent light from various other planes and increases clarity in the focal plane.[12]

In the present study, the mean values of the sealing ability of Group III (b), i.e., MTA plus with phytic acid was $(554.28 \pm 93.35)$ which showed the least microleakage values when compared to the other groups [Table 1]. Probable reason could be that Phytic acid has a strong binding affinity to important minerals, such as calcium, iron, and zinc. The binding of calcium with phytic acid is pH-dependent which leads to better chelating ability than conventional chelating agents. The results of this study were in harmony with the study done by Nassar et al.[11] In which 1% Phytic Acid was found to be more efficient in eliminating the smear layer as opposed to EDTA, which has a pH around 1.2 and this acidity, along with chelation ability, attributed to effective smear layer removal and Ca++ extraction.

However, the microleakage values of Group III (b) MTA Plus with Phytic acid $(554.28 \pm 93.35)$ was less numerically than Group III (a) Biodentine with Phytic acid $(590.80 \pm 74.95)$ which was not statistically significant with $P = 0.814$. This may be because at the intersection between material and root canal wall there is the development of the hydroxyapatite like crystals due to which the material prevents the penetration of dye leading to superior adhesion and least microleakage | Figures 1 and 2. Moreover, MTA being hydrophilic, when cured in moist environment results in setting expansion.[11]

The smear layer removing ability of Chitosan was more than EDTA but less than Phytic acid. This is in accordance with the literature that the Chitosan polymer which is hydrophilic favors intimate contact with root canal dentin; and adsorbs to root canal wall. It is important that the ionic interaction between the dentin calcium ions and the chelating agent takes place because it has large number of free hydroxyl and amino groups that makes it cationic in nature.[15] Moreover, amino groups
The present in polymer are protonated in acidic medium and attracts other molecules for adsorption to root dentin development and were capable of being delivered to deeper location of dentinal tubules. In addition, in the present study, as chitosan is insoluble in water it was dissolved in 1% acetic acid to form the solution, thus it was postulated that the acid might supplement the chelating efficacy of chitosan.

The our study mean values of Group I (a) Biodentine™ with 17% EDTA (825.29 ± 138.62) showed less amount of microleakage when compared to the mean values of Group I (b) MTA Plus® with 17% EDTA (963.74 ± 71.67) and was statistically significant with $P = 0.001$ and the removal of smear layer improved the marginal adaptation of Biodentine™. In the presence of physiological solution, Biodentine™ causes the uptake of Calcium and Silica in the root canal dentin. The rate of reaction of tricalcium silicate was higher for Biodentine™ than MTA owing to its optimized particle size distribution, the presence of Calcium Carbonate (CaCO$_3$) and the use of Calcium Chloride (CaCl$_2$). MTA which formed the hard tissue layer may be compared to the interfacial layer formed between Biodentine™ and dentine.

**CONCLUSIONS**

Within the limitations of this study conclusions drawn are as follows:

a. MTA Plus treated with 1% Phytic acid as smear layer removing agent showed the least microleakage followed by Biodentine treated with 1% Phytic acid.

b. There was a significant difference in microleakage between MTA Plus® and Biodentine™ when treated with 17% EDTA and 0.2% Chitosan as a smear layer removing agent.

c. MTA Plus treated with 17% EDTA showed the highest microleakage values when compared to other tested groups.

d. None of the materials tested in our study were free from microleakage, but we could safely conclude that smear layer removing agents aid in better adaptability of root end filling material.

This study was done in *in vitro* conditions, thus further long-term *in vivo* studies are required before setting up a Standard Operating Protocol.

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**Conflicts of interest**

There are no conflicts of interest.

**REFERENCES**

1. Girish CS, Ponnappa K, Girish T, Ponappa M. Sealing ability of mineral trioxide aggregate, calcium phosphate and polyacrylate particles in root end preparations using an Erbium: Yttriumaluminium garnet laser and ultrasonics evaluated by confocal laser scanning microscopy. J Conserv Dent 2013;16:304-8.
2. Torabinejad M, Higa RK, McKendry DJ, Pitt Ford TR. Dye leakage of four root end filling materials: Effects of blood contamination. J Endod 1994;20:159-63.
3. Gartner AH, Dorn SO. Advances in endodontic surgery. Dent Clin North Am 1992;36:357-78.
4. Roux D, Doméjean-Orliaguet S, Saade M. Leakage associated with intermediate restorative material and glass-ionomer cement retrograde fillings: A human and sheep teeth comparison with different aging procedures. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002;93:81-7.
5. Ingle JI, Bakland LK. Endodontics. 5th ed. London: B. C. Decker. Elsevier; 2002.
6. Torabinejad M, Hong CU, McDonald F, Pitt Ford TR. Physical and chemical properties of a new root-end filling material. JOE 1995;21:349-53.
7. Prati C, Gandolfi MG. Calcium silicate bioactive cements: Biological perspectives and clinical applications. Dent Mater 2015;31:351-70.
8. Laurent P, Camps J, About I. Biodentine™ induces TGFbeta1 release from human pulp cells and early dental pulp mineralization. Int Endod J 2012;45:439-48.
9. Bronnec F. Biodentine Active Biosilicate Technology Scientific File. Paris, France: Septodont; 2012.
10. Nikhil V, Jaiswal S, Bansal P, Arora R, Raj S, Malhotra P. Effect of phytic acid, ethylenediaminetetraacetic acid, and chitosan solutions on microhardness of the human radicular dentin. J Conserv Dent 2016;19:179-83.
11. Nassar M, Hiraiishi N, Tamura Y, Otsuki M, Aoki K, Tagami J. Phytic acid: An alternative root canal chelating agent. J Endod 2015;41:242-7.
12. Khandelwal A, Karthi J, Nadig R, Jain A. Sealing ability of mineral trioxide aggregate and Biodentine as the root end filling material, using two different retro preparation techniques – *An in vitro* study. Int J Contemp Dent Med Rev 2015;1:150115:1-6.
13. Mandava P, Bolla N, Thumu J, Vemuri S, Chukka S. Microleakage evaluation around retrograde filling materials prepared using conventional and ultrasonic techniques. J Clin Diagn Res 2015;9:ZC43-6.
14. Ravichandra PV, Vemisetty H, Deepthi K, Reddy S, Ramkriran D, Krishna MJ, et al. Comparative evaluation of marginal adaptation of Biodentine™ and other commonly used root end filling materials – *An in vitro* study. J Clin Diagn Res 2014;8:243-5.
15. Gerhards F, Wagner W. Sealing ability of five different retrograde filling materials. J Endod 1996;22:463-6.
16. Zhang J, Xia W, Liu P, Cheng Q, Tahiroiu T, Gu W, et al. Chitosan modification and pharmaceutical/biomedical applications. Mar Drugs 2010;8:1962-87.
17. Rhazi M, Desbrieres J, Tolaimate A, Rinaudo M, Vottero P, Alagui A, et al. Influence of the nature of the metal ions on the complexation with chitosan. Application to the treatment of liquid waste. Eur Polym J 2002;38:1523-6.
18. Santos AD, Moraes JC, Araujo EB, Yukimitsu K, Valério Filho WV. Physicochemical properties of MTA and a novel experimental cement. Int Endod J 2005;38:433-7.