Original Article

Comparative evaluation of apical microleakage of mineral trioxide aggregate, Biodentine, and Bio-C Repair as root-end filling materials using dye extraction method: An *in vitro* study

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Abstract

Context: Root-end filling materials enhance the apical sealing ability of the root canal to avoid leakage of irritants and to prevent the reentering of microorganisms.

Aim: The objective of the present study was to compare and evaluate apical microleakage in retrograde filling materials with mineral trioxide aggregate (MTA), Biodentine, and Bio-C Repair using an ultraviolet (UV)–visible spectrophotometer.

Materials and Methods: Fifty maxillary incisors were selected and decoronated. Instrumentation was done with ProTaper rotary files and obturated with AH Plus sealer and ProTaper gutta-percha cone using lateral condensation technique. The apical part of each root was resected at 90° to the long axis of the root for 3 mm, and retrograde cavity preparation was done up to 3 mm using an ultrasonic retro tip. After conditioning the root end with 17% ethylenediaminetetraacetic acid, the teeth were divided into four groups. Group 1 = Positive Control (n = 5), Group 2 was filled with MTA (n = 15), Group 3 was filled with Biodentine (n = 15), and Group 4 was filled with Bio-C Repair (n = 15). All samples were incubated in 5 mL of 2% methylene blue dye for 72 h after which teeth were immersed in 65% nitric acid for 72 h. The solutions were then filtered using fine grit filter paper and centrifuged at 3500 rpm for 5 min. The solution thus collected was used to determine absorbency in UV–visible spectrophotometer at 550 nm.

Statistical Analysis: The data were analyzed using one-way analysis of variance and Tukey *post hoc* tests. $P < 0.05$ was considered significant for all analyses.

Results: The results showed that Biodentine had the least dye absorbance, which means less microleakage, and there was no significant difference between MTA and Bio-C Repair.

Conclusion: Biodentine showed superior sealing ability as a retrograde filling material compared to Bio-C Repair and MTA.

Keywords: Bio-C Repair; biodentine; dye extraction; root-end filling

INTRODUCTION

Endodontic treatment success is achieved by the elimination of the microorganisms from the root canal system and the

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development of fluid tight seal using a material of adequate compatibility.[1] Despite the new endodontic techniques, effective materials, and instruments, the resolution of periapical pathosis is not achieved in certain cases where surgical endodontic intervention is needed.[2]

Teeth with persistent periapical lesions, in which root canal retreatment has failed or is not feasible are salvaged by apicoectomy, an established surgical procedure. The procedure involves exposure of the root apex, curettage of the lesion, root-end resection, root-end cavity preparation, and root-end filling.[3]

The concern of apicoectomy is not only the removal of diseased periapical tissue and root apex but also the resealing of the root canal system with suitable root-end filling material.[4]

The properties of root-end filling material are critical in determining the success of peri-radicular surgery. Inappropriate marginal sealing of retrocavity may allow the percolation of microorganisms and their products between the root canal system and peri-radicular tissues, thus leading to treatment failure.[5]

Apical microleakage continues to be a topic of interest; despite advances in endodontics, clinical failure still occurs. Gutta-Percha, Amalgam, Cavit, Intermediate Restorative Material, Super EBA, Diaket, Glass Ionomer cement, Composite Resins, Zinc Oxide Eugenol cement, etc., have been used traditionally as a root end-filling material.[6,7]

Later, mineral trioxide aggregate (MTA) was developed, which emerged as an ideal root-end filling material. MTA is considered the gold standard, and other newer materials are compared with it because of its osseoconductive, osseoinductive, bioactive properties, and good marginal seal. Aqrabawi[8] compared the sealing ability of amalgam, super-EBA cement, and MTA as retrograde filling material and showed that MTA provides a better seal than other materials. However, the study conducted by Mandava *et al*.[9] and evaluated the apical microleakage of root-end cavities filled with MTA, Biodentine, and LC GIC using two different cavity preparation techniques that are conventional bur preparation and ultrasonic tip preparation. The result of their study showed significantly less microleakage of MTA compared to Biodentine and LC GIC.

A new tricalcium silicate-based cement material, Biodentine was introduced in 2009. It has better handling properties and a shorter setting time than MTA. In addition, it possesses very similar physical properties to dentin and poses a low risk of tooth discoloration. Kokate and Pawar[10] compared the microleakage of MTA, GIC, and Biodentine using the dye penetration method under a stereomicroscope. The results of their study showed that there was significantly less leakage in Biodentine when compared to MTA and GIC.

Bio-C Repair is a bioceramic ready-to-use putty-like repair cement composed of tri and dicalcium silicates, tricalcium aluminate, calcium oxide, and zirconium oxide. It can be used as root-end filling material in apicoectomy cases because of its high alkalinity (bactericidal), bioactivity, and chemical adhesion to dentin (prevents bacterial infiltration), setting expansion.[11,12]

Ideally, the placement of filling material should be carried out in a moisture-free environment. However, in a clinical surgical scenario, there is a high chance of contamination through blood or saliva.[13] Hence, the sealing ability of retrograde filling materials plays a crucial role in such conditions and determines the long-term success of such treatment.

As there are no studies done till date on the comparison of microleakage between MTA, Biodentine, and Bio-C Repair. The present study compared the microleakage between MTA, Biodentine, and Bio-C Repair using an ultraviolet (UV)–visible spectrophotometer.

MATERIALS AND METHODS

This study was approved by the Scientific and Ethical Committee (Ref No 33/IEC/LIDS/2023). Fifty maxillary incisors that were extracted for periodontal reasons were selected for the study. Criteria for tooth selection include a single root without curvature, no visible root caries, fracture, or cracks. The roots were cleaned of attached tissues and calculus, washed, and debrided with 5.25% sodium hypochlorite (NaOCl) (Prime Dental, Thane, India), and stored in normal saline until use.

All teeth were de-coronated at the length of 14 mm from the apex with the help of a diamond disc (Kerr Dental Products, California, USA). Working length was determined using a 15 kK file (Mani, Inc., Tochigi, Japan) [Figure 1]. Chemomechanical preparation was done till F3 (Protaper) using an E Connect Endomotor, with copious irrigation with saline, 5.25% NaOCL, 17% ethylenediaminetetraacetic acid (Prevest Denpro, India), and 2% chlorhexidine. After the preparation, the root canal was thoroughly dried with paper points and obturation was done with lateral compaction technique using gutta-percha and AH Plus sealer (Maillefer, Dentsply). Apical 3 mm was measured, and the root end was resected perpendicular to the tooth's long axis with a diamond disc. The root end cavity at a depth of 3 mm was prepared by an ultrasonic retro tip (AS3D-Satelec). Samples were air-dried and divided into four groups according to the root end-filling material used, Group 1: Control (n = 5), Group 2: MTA (Angelus, Londrina, Brazil) ($n = 15$), Group 3: Biodentine (Septodont, Saint-Maur-des-Fosses, France) ($n = 15$), and Group 4: Bio-C Repair (Angelus, Londrina, Brazil) ($n = 15$).

Samples were coated with two coats of nail varnish, leaving apical 3 mm, and then immersed in 5 mL of 2% methylene blue dye (Sigma Aldrich) for 72 h. Samples were removed from the dye, and nail polish was removed and rinsed for 10 min using distilled water. Each sample was immersed in the test tube containing 5-ml nitric acid for 48 h. The obtained solution was centrifuged at 3500 rpm for 5 min. 4-mL supernatant liquid was then analyzed in an UV spectrophotometer (PerkinElmer Lambda 25, Norwalk, USA) at 550-nm wavelength with concentrated nitric acid as the blank, and the readings were recorded as absorbance units.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 20.00 (IBM SPSS, IBM Corp., Armonk, NY, USA) to compare the mean apical microleakage of the groups and determine the significance of differences between different groups. One-way analysis of variance (ANOVA) with Tukey's *post hoc* tests was used to analyze the study data.

Table 1: Comparison of four groups with mean microleakage by one-way analysis of variance

Group	*n*	Mean	SD	*P*
Group 1 (control)	5	1.0924	0.41679	0.000 HS
Group 2 (MTA)	15	0.3279	0.07455	
Group 3 (Biodentine)	15	0.1606	0.06901	
Group 4 (Bio-C Repair)	15	0.3195	0.12707	

HS: Highly significant at $P<0.01$, MTA: Mineral trioxide aggregate, SD: Standard deviation

Table 2: Tukey's *post hoc* analysis (multiple comparisons) of mean values of absorbance

Group	Mean difference	*P*
Control versus MTA	0.76457*	0.000 HS
Control versus Biodentine	0.93185*	0.000 HS
Control versus Bio-C Repair	0.77292*	0.000 HS
MTA versus Biodentine	0.16728*	0.021 S
MTA versus Bio-C Repair	0.00835	0.999
Biodentine versus Bio-C Repair	0.15893*	0.031 S

*HS: Highly significant at $P<0.01$, S: Significant at $P<0.05$, MTA: Mineral trioxide aggregate

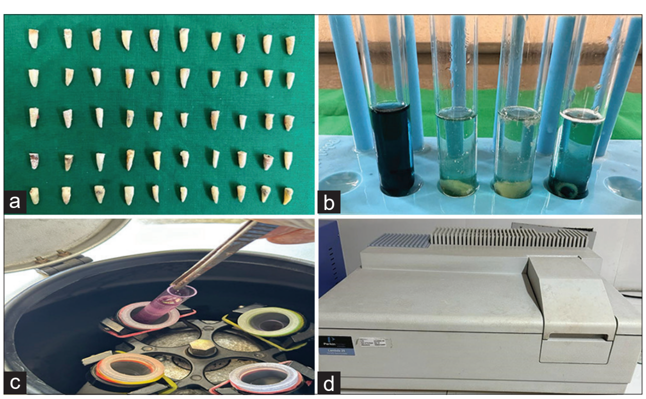

Figure 1: Decoronated samples leaving 14 mm of tooth length (a), sample immersed in conc 65% nitric acid (b), centrifugation of the samples (c), ultraviolet–visible spectrophotometer (d)

RESULTS

Table 1 and Figure 2 show a comparison of four groups with mean microleakage by one-way ANOVA test revealed that Biodentine shows the least dye absorbance, and there was no significant difference between MTA and Bio-C Repair with mean microleakage values. Table 2 shows the results of Tukey's *post hoc* analysis and suggests that there is a significant difference between all groups except Group 2 (MTA) and Group 4 (Bio-C Repair).

DISCUSSION

The goal of a periradicular surgery is to gain access to the affected area, evaluate the root circumference and root canal anatomy, and placement of a biocompatible root-end filling that seals and stimulates the regeneration of periodontium. Numerous substances have been used as root-end filling materials. The choice of a root-end filling material could be governed by handling properties, biocompatibility, apical seal, and long-term clinical success.[14]

Inadequate apical seal leads to microleakage and is one of the major causes of surgical endodontic failure. Microleakage is defined as the movement of bacteria, fluids, molecules, or ions between the tooth and restorations of any type.[15] Various techniques for assessing microleakage have been developed and utilized. Most modern techniques utilize different principles involving biological, chemical, electrical, physical, or radioactive components.[16]

These include the use of dyes, radioactive isotopes, air pressure, fluid filtration, bacteria, neutron activation analysis, artificial caries, scanning electron microscopy, calcium hydroxide, and other methods.

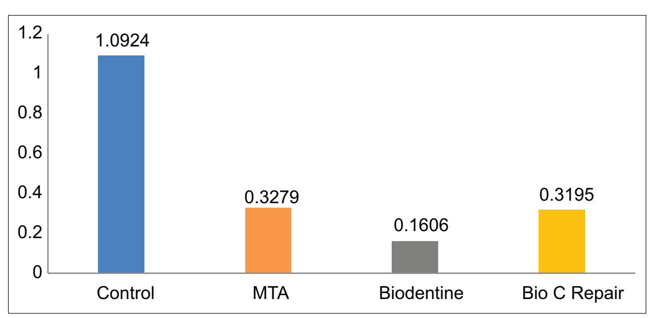

Figure 2: Comparison of mean microleakage values of root-end filling materials

Dye extraction method was used in the present study. In the dye extraction method, the teeth were dissolved in acids that release all the dye from the interface, after which an UV-spectrophotometer measured the optical density of the obtained solution. In the present study, the results were recorded as a measure of the absorbance of light.

According to the Beer-Lamberts law, the absorbency of the solution is directly proportional to the concentration of absorbing species in the solution and path length. UV–visible spectroscopy can be used to determine the concentration of the absorber in the solution for a fixed path length. Hence, the absorbance of the solution is directly related to the amount of microleakage.[17,18] The apical microleakage was calculated by measuring the absorbance of the solution in an UV–visible spectrophotometer.

Maxillary incisors were selected to simulate the clinical scenario and to obtain predictable results. Kim and Kratchman suggested removing at least 3 mm of the root end which reduces 98% of the apical ramifications and 93% of the lateral canals.[19] They also proposed that root end amputation of <3 mm may lead to further infection due to incomplete removal of apical ramifications and lateral canals.

The least microleakage was exhibited by Biodentine (0.1606) among all the groups. The result of our study is in concurrence with the study conducted by Khandelwal *et al*.,[20] Radeva *et al*.,[21] and Naik *et al*.,[22] comparing the sealing ability of MTA and Biodentine as root-end filling material. Khandelwal *et al*.[20] concluded that Biodentine can be used as a replacement for MTA.

The study by Radeva *et al*.[21] concluded that Biodentine can be more effective as an apical sealing material compared to MTA. Naik *et al*.[22] concluded that the apical seal obtained with Biodentine was superior to that obtained with MTA.

Biodentine is a bioactive dentin substitute designed as a dentin replacement material. The powder component of the material consists of tricalcium silicate, dicalcium silicate, calcium carbonate and oxide filler, iron oxide shade, and zirconium oxide, respectively, whereas zirconium oxide serves as a water-reducing agent. Liquid consists of calcium chloride as an accelerator and hydrosoluble polymer which acts as water-reducing agent. The presence of a setting accelerator in Biodentine results in faster setting, thereby improving its handling properties and strength.

Biodentine was chosen as a retrograde filling material in this study. Hindlekar and Raghavendra in their study stated that the tricalcium oxide in the cement of Biodentine reacts with the tissue fluid and stimulates dentin regeneration by inducing odontoblast differentiation from pulp progenitor cells.[23] Further, Malhotra and Hegde proposed that the smaller size of Biodentine particles aids in enhanced adaptation at the cavity surface and filling interface.[24] The decreased pore volume and porosity of Biodentine as compared to MTA resulted in better sealing ability.[25] The modified composition of the Biodentine powder such as the absence of calcium aluminate, calcium sulfate, and the presence of calcium chloride in liquid has improved its physical properties mainly handling and sealing ability. The faster setting of Biodentine would have prevented the prolonged leakage, thereby reducing the bacterial contamination.

This material can also stimulate cell growth and induce hydroxyapatite (HA) formation on the surface of the material when exposed to simulated body fluid.[26] HA has been shown to induce bone formation, growth, and maintenance at the bone material interface.[27] The thickness of the calcium- and silicon-rich layers increased over time, and the thickness of the calcium- and silicon-rich layer was significantly larger in Biodentine.[28] The biomineralization ability of Biodentine, most likely through the formation of tags, greater calcium and silicon uptake from adjacent root canal dentin and least microleakage when compared with other filling materials are the probable reasons for its least dye absorbance.

MTA (0.3279) shows mean microleakage value similar with Bio-C repair (0.3195), the possible reason could be, in MTA, the powder particles are hydrophilic and small, when they come in contact with the moisture the hydration reaction occurs that results in colloidal gel structure which solidifies in the mineralized tooth structure can result in decrease in leakage.[29]

In the current study, Bio-C Repair (0.3195) shows a mean microleakage value when compared with Biodentine (0.1606) and shows a mean microleakage value similar to MTA (0.3279). Bio-C Repair is cement with the considerable advantage of being supplied in a ready-to-use form (putty), leading to better handling properties compared to Biodentine and MTA. Bio-C Repair was found to mainly be composed of carbon (34.81%) and oxygen (34.51%), with a lower concentration of calcium compared to the other biomaterials, which mainly contain oxygen and calcium.

The marginal adaptation and sealing ability of Bio-C Repair have been evaluated by Sunanda *et al*.[30] state that the low solubility and volumetric loss, in addition to the dimensional expansion demonstrated by Bio-C Repair, may be related to its hydration, water sorption, and particle size.

Limitations of this *in vitro* study include that the study has not evaluated the effect of moisture contamination on apical microleakage.

CONCLUSION

The current study concluded that all retrograde filling materials used in the study such as MTA, Biodentine, and Bio-C Repair showed some amount of microleakage, in which Biodentine shows less microleakage compared to Bio-C Repair and MTA.

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Conflicts of interest
There are no conflicts of interest.

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