ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 11, Issue, 08, pp.6553-6558, August, 2019

DOI: https://doi.org/10.24941/ijcr.36440.08.2019

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

COMPARATIVE EVALUATION OF SEALING ABILITY OF ENDOSEQUENCE, MTA AND BIODENTINE AS RETROGRADE FILLING MATERIAL – AN IN VITRO STUDY

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ARTICLE INFO

Article History:
Received 11th May, 2019
Received in revised form
17th June, 2019
Accepted 14th July, 2019
Published online 31st August, 2019

Key Words:
Sealing Ability,
Microleakage,
Endosequence,
Biodentine,
MTA.

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ABSTRACT

Introduction: The success of periapical surgery is dictated by elimination of infected tissues and adequate apical seal. Among the various materials tested, MTA has shown good sealing ability and biocompatibility in previous studies. Materials like Biodentine, ESBCRRM have been introduced with the aim of overcoming some of the disadvantages of the MTA. Hence the aim of this study was to evaluate the sealing ability of Biodentine, MTA and Endosequence, as a root end filling material, using stereomicroscope. ***Aim****:* To Compare and evaluate the sealing ability of MTA, Endosequence and Biodentine as retrograde filling material. ***Methodology:*** Sixty extracted maxillary permanent incisors were selected and stored in normal saline. The coronal portion was sectioned at CEJ access cavities were prepared, The working length was determined, BMP was done. Obturation done with gutta-percha. All sample were stored at 37 ± 1°C and 100% relative humidity for 7 days in incubator. The obturated samples were randomly divided into 5 groups.12 samples each group 1-negative control, group 2- positive control, group 3- mta, group 4- endosequence, group 5- biodentine. The apical 3mm of each root were sectioned. Round bur was used to prepare a 3mm root end preparation in all teeth. Apical leakage was evaluated using Rhodamine B dye and measured stereomicroscope. Mean and standard deviation was performed using a one - way ANOVA analysis of variance. ***Result:*** There was significantly less microleakage in Group - IV (endosequence) when compared to Group - V (Biodentine) and Group - III (MTA), but there was no significant difference between Group - V (Biodentine) and Group - III (MTA). ***Conclusion****:* On comparative evaluation of results of this in vitro study, it was concluded that ES-BCRR, Biodentine& MTA exhibited microleakage with Group II (ES-BCRR) showing the least microleakage of all.

Citation: Dr. Harshita Pathak, Dr. Anubhuti. Dr. Priyanka Kumari and Dr. Ravi Raj, **2019.** "Comparative Evaluation of Sealing Ability of Endosequence, MTA and Biodentine as Retrograde Filling Material – An In vitro Study", *International Journal of Current Research*, 11, (08), 6553-6558.

INTRODUCTION

The objectives of modern endodontic therapy are to clean and shape the root canal system removing all organic material and sealing the root canal with a three-dimensional filling. Pulpal and periradicular pathosis develop more frequently due to bacterial contamination of the pulp and periradicular tissues. The removal of irritant factors like bacteria and adequate obturation of the root canal system after thorough cleaning and shaping results in the resolution of periradicular lesions[1].Although the degree of success following root canal therapy has been reported to be as high as 98.7%, the majority of the failure in the case of conventional root canal therapy is due to inadequate apical seal[2]. The surgical approach is indicated when healing is not achieved after non-surgical endodontic therapy, or when re-treatment is not possible or has failed[1]. The procedure routinely consists of the exposure and resection of the involved root apex, followed by the insertion of a root-end filling material[1,3]. The root-end filling material should improve the sealing of the existing root canal filling or provide an apical seal to an otherwise unobturated root canal, thus preventing the movement of bacteria and bacterial products from the root canal system to periapical tissues[3,4].These retrofilling materials should be biocompatible, easy to use and should not be sensitive to moisture[5]. Many materials have been investigated in an attempt to achieve the most effective seal when used as retrograde filling[6]. A novel material, mineral trioxide aggregate (MTA) was reported to seal off all the communication between the root canal system and external surface of the tooth[7]. MTA has various advantages such as biocompatibility, excellent sealing ability, radio-opacity and the ability to set in moist environments, but it also possesses disadvantages like long setting time, difficulty

in manipulating the material and high cost. This has led to researchers searching for other suitable materials[8]. More recently, a new calcium-silicate restorative material called Biodentine has been produced by Septodont (Saint Maurdes Fosses, France). It can be used not only as an endodontic repair material like MTA but also as a coronal restorative material for dentine replacement[9]. It can also be used in root-end filling, repair of root and furcation perforations, apical plugs, apexification and direct & indirect pulp capping[10]. Also recently, a new material called Endosequence Bioceramic Root Repair Material (ES-BCRR; Brasseler USA) has been introduced to be used as a root-end filling material as well as a root repair material[11].This material is available as a syringable paste and as a root-end filling due to the ease of handling the material and the elimination of the need to mix to a proper consistency[11].

The quality of apical seal achieved by root end filling material has been assessed by various means like the degree of dye penetration, radioisotope, penetration, bacterial penetration, electro chemical means and fluid filtration techniques[12].The dye penetration method for measuring method used for measuring sealing ability is the most popular.Various dyes that can be used include India ink, basic fuchsin, silver nitrate with developer and methylene blue. According to the various studies conducted, methylene blue has been proved to be a useful aid in endodontics[13]. There are fewer studies which evaluated the apical sealing ability of these new materials. The aim of this in vitro pilot study was to compare the microleakage of three different root end filling materials MTA, Biodentine and newly researched Endosequence Bioceramic Root Repair material using dye penetration method under stereomicroscope.

MATERIALS AND METHODS

Seventy five recently extracted human maxillary permanent incisors were selected for the experiment. The teeth had been extracted for periodontal and orthodontic reasons. Following extraction, the teeth were cleaned and stored in Formalin. All the teeth were examined with magnifying glass with 4x magnification. Preoperative radiographs were exposed to confirm the canal anatomy. The coronal portion of the selected teeth was sectioned at CEJ using a diamond disc. After access cavities were prepared with size #2 bur, a size 10 K-file (Sybron endo) was introduced into the canal until the tip was visible at the apical foramen. The working length was determined by subtracting 0.5 mm from this measurement. Coronal enlargement was done with Gates Glidden drill of size. Root canals were prepared using crown down technique till 25/0.06 K3 XF(Sybron Endo). The canals were irrigated between instruments with Normal Saline and 2 ml of 5.25% Sodium hypochlorite (NaOCI). Subsequently, root canals were dried and obturated with guttapercha and AH Plus sealer (Dentsply, Malliefer) using lateral compaction technique. The canal orifices were sealed with Glass ionomer cement. All samples were stored at 37 ± 1°C and 100% relative humidity for 7 days in an incubator. The apical 3 mm of the obturated roots in the other groups were resected at the apical end at 90° to the long axis using tapered fissure bur. A standardized 3 mm deep and 1.2 mm wide root-end cavity was prepared using a round bur and undercut was made with inverted cone bur following the morphology of root canal. The cavities were irrigated with EDTA which was followed by saline, and the cavity was then dried.

Group 1, white MTA (ProRoot MTA, Dentsply Tulsa Dental) root end filling (n=12):MTA was mixed according to manufacturer's instructions and incrementally placed into the root-end preparation using MTA system carrier (GDC) and condensed using Buchanan condensers (Sybron Endo). Following the initial set of the MTA the samples were then stored in 2" x 2" gauze moistened with sterile saline and placed in an incubator at 37°C for 48 hours to allow for final setting of the MTA.

Group 2, ES-BCRR (Brasseler USA) root end filling (n=12):ES-BCRR injectable was incrementally placed into the root-end preparation according to the manufacturer's directions and condensed using Buchanan condensers. Following the initial set of ES-BCRR the samples were then stored in 2" x 2" gauze moistened with sterile saline and placed in an incubator at 37°C for 48 hours to allow for final setting of the ES-BCRR.

Group 3, Biodentine (Septodont, Saint Maurdes Fosses, France) root end filling (n=12):Biodentine was mixed according to manufacturer's instructions and incrementally into the root-end preparation using MTA system carrier (GDC) and condensed using Buchanan condensers.Following the initial set of the Biodentine the samples were then stored in 2" x 2" gauze moistened with sterile saline and placed in an incubator at 37°C for 48 hours to allow for final setting of the Biodentine.

Group 4, the negative control (n=12), apical preparation was not done.Thesamples were then stored in 2" x 2" gauze moistened with sterile saline and placed in an incubator at 37°C for 48 hours.

Group 5, positive control (n=12): Teeth were not filled with any root end filling material. Following this procedure, the samples were stored in moist 2" x 2" gauze moistened with sterile saline and placed in an incubator at 37°C for 48 hours.
Apical leakage was evaluated using dye penetration technique. Following root end filling, all the samples in groups I ,II, III & V were then coated with three layers of nail varnish except at the apical resected root surface & root - end filling, and then allowed to dry.

In Group IV (Negative control) the entire specimen including the root canal orifice and the apical foramen were coated with three of layers of nail polish in order to prevent leakage in the root canal system.

All the samples from all groups were suspended in 2% Methylene blue dye (Merck Ltd., Mumbai) solution for 72 hours at 37°C and 100% humidity. Thereafter, the samples were removed, rinsed for 15 minutes under tap running water, and air dried. Nail varnish was removed with a scalpel and samples were sectioned vertically in a bucco-lingual direction into two sections with a diamond disc under copious irrigation with cold water. Dye penetration was measured linearly to its further extent within the root end cavity using a calibrated stereomicroscope (Motic, Hong Kong) with 30X magnification. The greatest depth of dye penetration along one of the cavity walls was measured in millimetres. Data was entered in Microsoft excel 2016 for Windows. Mean, standard deviation (SD), minimum and maximum values of micro leakage in different groups were calculated. The parametric test, One-way ANOVA was applied for comparison of micro leakage in between different groups.

RESULTS

There was significantly less microleakage in Group - IV (endosequence) when compared to Group - V (Biodentine) and Group - III (MTA), but there was no significant difference between Group - V (Biodentine) and Group - III (MTA).

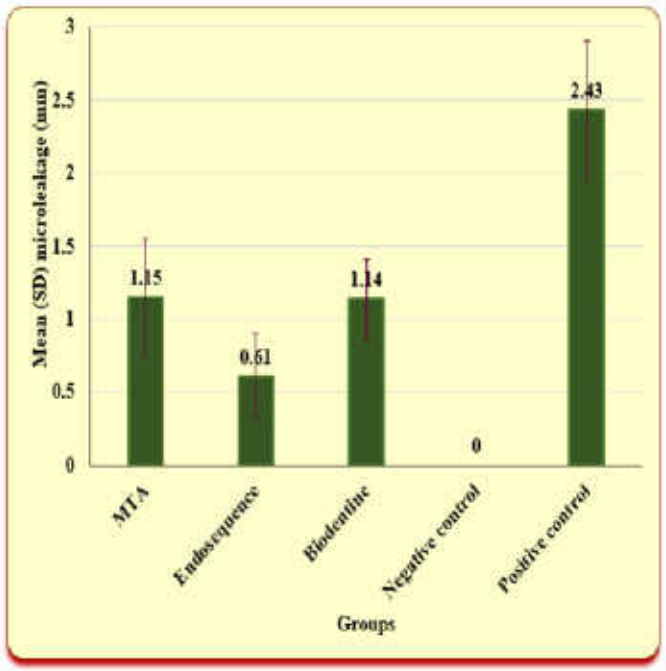
TABLE 6: MEAN, STANDARD DEVIATION (SD), MINIMUM AND MAXIMUM VALUES OF MICROLEAKAGE IN DIFFERENT GROUPS

Groups		Microleakage (mm)	
		Mean ± SD	Min-Max
Group 1	MTA (n =15)	1.15 ± 0.40	0.40-1.60
Group 2	Endosequence (n =15)	0.61 ± 0.29	0.20-1.00
Group 3	Biodentine (n =15)	1.14 ± 0.27	0.60-1.50
Group 4	Negative control (n =15)	0.00 ± 0.00	0.00-0.00
Group 5	Positive control (n =15)	2.43 ± 0.48	1.70-3.00
One-way ANOVA		F = 110.435	
P value		0.000 (<0.001), Highly significant	

TABLE 7: PAIRWISE COMPARISON OF MICROLEAKAGE IN DIFFERENT GROUPS USING TUKEY POST HOC TEST

Comparison groups (Pairs)	Mean difference (mm)	P value
MTA and Endosequence	0.54	0.000 (<0.001), Highly significant
MTA and Biodentine	0.01	1.000 (>0.05), Not significant
MTA and Negative control	1.15	0.000 (<0.001), Highly significant
MTA and Positive control	-1.27	0.000 (<0.001), Highly significant
Endosequence and Biodentine	-0.53	0.000 (<0.001), Highly significant
Endosequence and Negative control	0.61	0.000 (<0.001), Highly significant
Endosequence and Positive control	-1.81	0.000 (<0.001), Highly significant
Biodentine and Negative control	1.14	0.000 (<0.001), Highly significant
Biodentine and Positive control	-1.29	0.000 (<0.001), Highly significant
Negative control and Positive control	-2.43	0.000 (<0.001), Highly significant

GRAPH 3: MEAN AND STANDARD DEVIATION (SD) VALUES OF MICROLEAKAGE IN DIFFERENT GROUPS



GRAPH 4: PAIRWISE COMPARISON OF MICROLEAKAGE IN DIFFERENT GROUPS

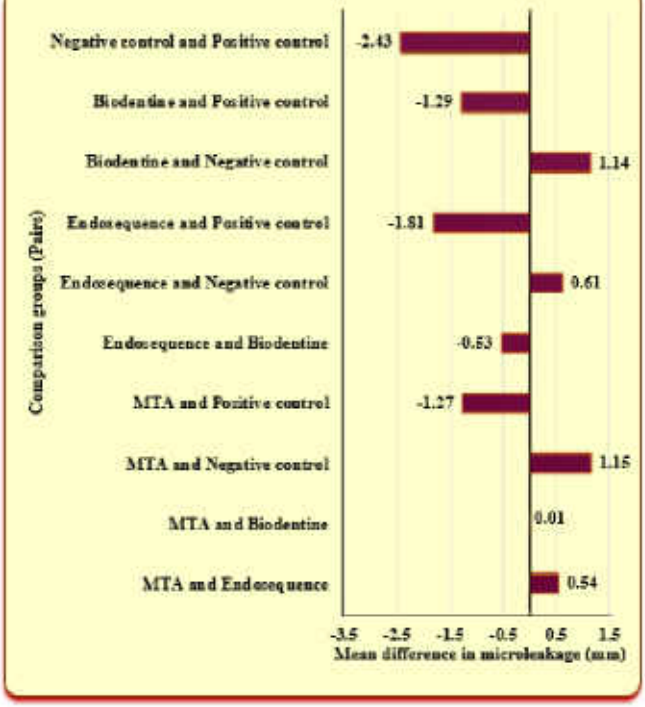


Figure 1. Radiograph of obturated tooth

Figure 2. Root-end resection

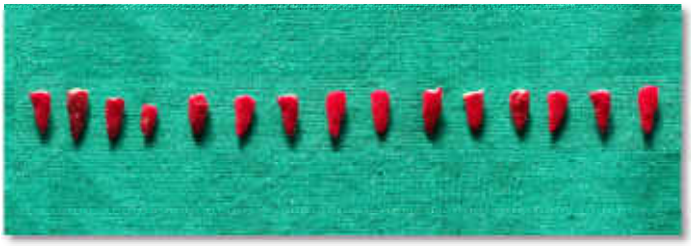


Figure 3. Nail varnish applied on the prepared samples

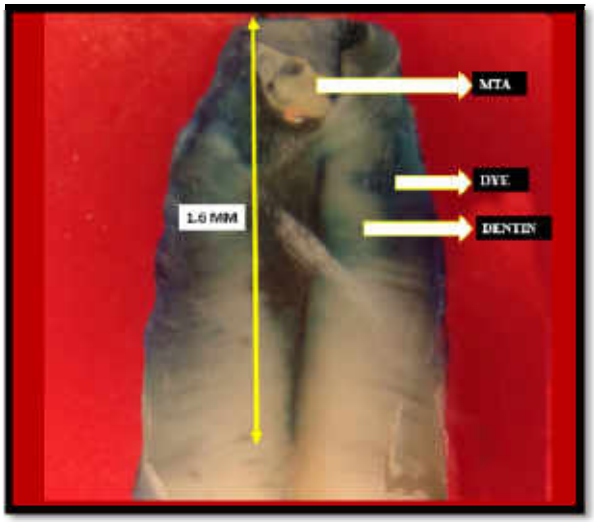


Figure 4. Microleakage in MTA

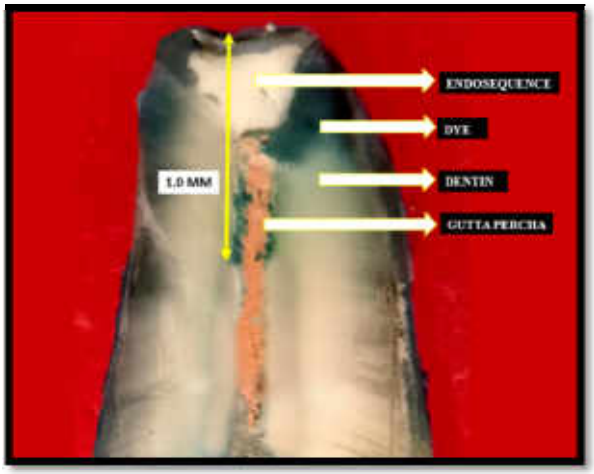


Figure 4. Microleakage inEndosequence

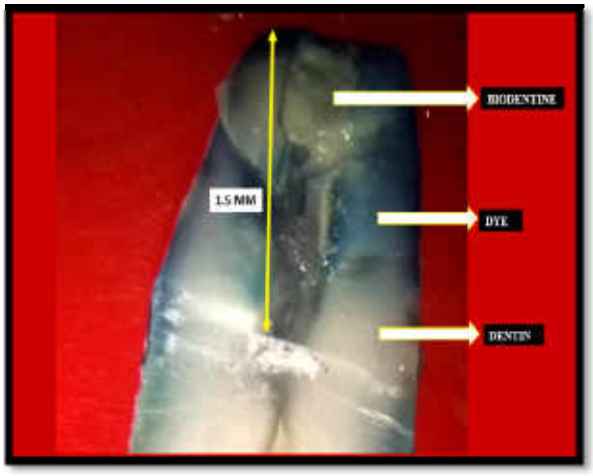


Figure 5. Microleakage in Biodentine

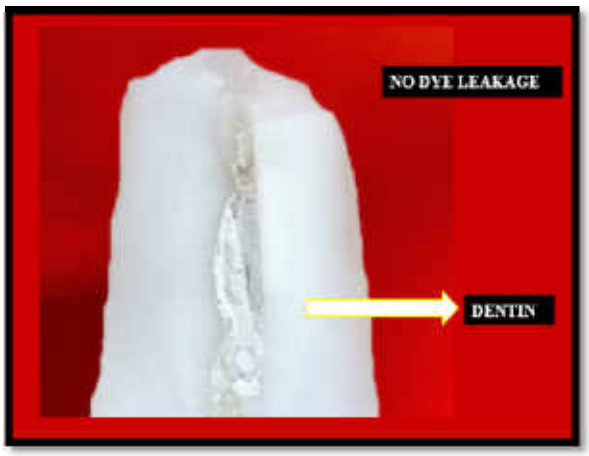


Figure 6. No Microleakage in negative control

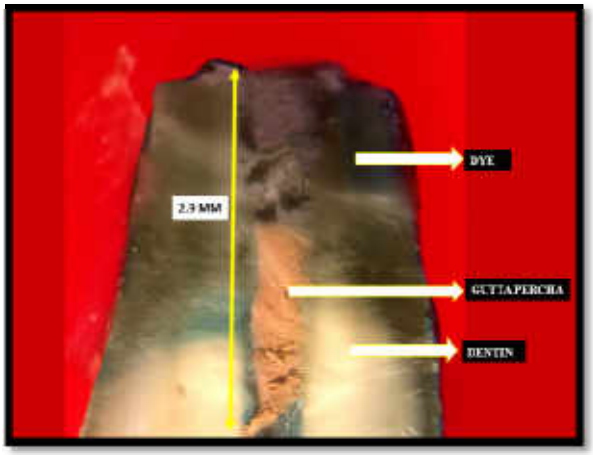


Figure 7. No Microleakage in positive control

DISCUSSION

The development or persistence of a periapical radiolucency following endodontic treatment associated with clinical signs and symptoms of periapical infection is often regarded criterion of failure[14]. In such cases decision must be made to choose between non-surgical retreatment and surgical treatment to retain the teeth. Information on treatment outcomes is essential for the decision-making process[15].In cases where retreatment is not possible or has failed, the surgical approach is indicated[16].The goal of periradicular surgery is to gain access to the affected area, evaluate the root circumference and root canal anatomy and place a biocompatible seal in the form of root end filling that stimulates the regeneration of periodontium[17]. Endodontic surgery includes three critical steps to eliminate endodontic pathogens. These steps include surgical debridement of pathological periradicular tissue, root-end resection and retrograde root canal obturation (root-end filling)[16]. The purpose of root-end filling is to establish an apical seal of the resected root[14].The selection of appropriate retrograde filling material is critical for insuring favourable outcome of endodontic surgery[18]. An ideal endodontic root-end filling material should show good biocompatibility with host tissue, excellent apical sealing, insoluble in tissue fluids, dimensionally stable, unaffected by moisture during setting, should prevent leakage of bacteria and their by-products into periradicular tissues, nontoxic, noncarcinogenic, radiopaque, easy handling, low cost, and should have long term clinical success[2,17,19]. With new materials being constantly introduced,

it is necessary to collect clinical evidence on the performances of these materials to help the clinicians and the patients to make an informed decision[15]. Several methods have been used to assess microleakage. These include methods such as fluid filtration, dye penetration, dye extraction, bacterial and protein leakage models. Recent methods include by using radioactive isotopes, artificial caries, scanning electron microscope, neutron activation analysis, and electrical conductivity[20,21]. In the current study, methylene blue dye penetration method was selected to study microleakage because it is inexpensive and easy to manipulate, as well as it has a high degree of staining and molecular weight even lower than that of bacterial toxins[22]. Over the years, a wide variety of materials have been advocated for use as root end filling materials till now such as silver amalgam, gold foil, zinc oxide eugenol cements (IRM and Super EBA), glass ionomer cement, Composite resins, resin-glass ionomer hybrids and Mineral trioxide aggregate[8]. "However to date, no material has been found to satisfy all the requirements of an ideal root-end filling material[23]. Mineral trioxide aggregate (MTA) was introduced by Mohmoud Torabinejad at Loma Linda university, California, USA in 1993[24] and was given approval for endodontic use by the U.S. Food and Drug Administration in 1998[25]. It is a unique material with several clinical applications[26]. It was introduced as root end filling material. Its major constituents are tricalcium silicate, tricalcium aluminate, tricalcium oxide, silicate oxide, bismuth oxide, calcium carbonate[8]. It has favourable properties suitable for root end filling material such as excellent sealing ability, biocompatibility[27], good compressive strength (67Mpa), insoluble in fluids once set, radiopacity and antibacterial effect[28]. Torabinejad *et al.* (1995) concluded that MTA is potential to activate the cementoblasts and eventually cementum production[29]. It also allows the overgrowth of PDL fibre over its surface[29]. Despite of its favourable properties, MTA has certain drawbacks like prolong setting time (2h 45mins) which might contribute to leakage, surface disintegration, loss of marginal adaptation and continuity of the material, difficulty in manipulation, technique sensitive and it is quite expensive as well[30,31,32].

These disadvantages led to the development of new materials such as Biodentine (Septodont, France), Endosequence Bioceramic Root Repair Material (Brasseler, USA). Biodentine is a calcium silicate based restorative material[33] also known as "dentine in a capsule"[34]. It is a bioactive cement with dentine like mechanical properties and has beneficial effect on living cells and acts in a biocompatible manner[35].Powder is composed of tricalcium silicate, dicalcium silicate, calcium carbonate, zirconium dioxide[8]. In liquid calcium chloride is added in aqueous solution to increase its setting time[8]. Both of them are mixed in triturator for 30 seconds prior to insertion. It sets in about 12 minutes[8]. The consistency of Biodentine is similar to that of phosphate cement[8]. Endosequencebioceramic root repair material (ES-BCRR, Brasseler, USA) has been introduced[11]. It is bioceramic material, available as premixed syringe form and is composed of zirconium oxide, calcium silicates, tantalum oxide, calcium phosphate monobasic, thickening agents and proprietary fillers[36]. EC-BCRR is manufactured in a syringeable form which is flowable and a putty form, which is firm and moldable[11].The manufacturers of Endosequence material claim that premixed. Endoseuqence has a working time of approximately 30+ minutes, a setting reaction initiated by moisture and a final set achieved approximately 4 hours later with calcium silicate portion of material produces a calcium silicate hydrate gel and calcium hydroxide[30]. The calcium hydroxide then interacts with phosphate ion to form hydroxyapatite and water. The water produced continues to react with calcium silicates to precipitate additional gel like calcium silicate hydrate[30]. According to developers of the Endosequence, the water supplied through this reaction is an important factor in controlling the hydration rate and the setting time of this material[11]. EC-BCRR has shown promising biological and physical properties as a new root end filling material. As there are limited literature available which compared the apical sealing ability of these materials this study was undertaken. Among all the materials used in this study Group II (ES-BCRR) showed minimum amount of apical dye leakage i.e. 0.61 ± 0.29 mm when compared with Group I (MTA) in which mean dye leakage was 1.15 ± 0.04 mm and Group III (Biodentine) in which mean dye leakage was 1.14 ± 0.27.The significant difference between in the amount of dye leakage between Group II (Endosequence) and Group I (MTA) & Group III (Biodentine) is may be due to ES-BCRR comes premixed with an intermediate restorative material like consistency and therefore, is easy to handle. Its particle size, which allows the premixed material to penetrate into the dentinal tubules and bond to adjacent dentine. Endosequence is directly applied over the prepared cavity and the by-products formed in the setting reaction of are hydroxyapatite and water. Studies have shown that the material sets at a highly alkaline pH and has antibacterial activity. According to the manufacturers of Endosequence, water formed in this reaction is important in controlling hydration rate and setting reaction of this material. Bioceramics have the advantage of forming hydroxyapatite and ultimately a bond between dentine and filling materia. This may be the reason why there was significant less microleakage in ES-BCRR when compared with the other two materials. In the present study no significant difference in mean microleakage values were observed between Group III i.e. Biodentine and Group I i.e. MTA. The difference was 0.01mm. This difference in these two groups is may be due to the similar properties they have.

Conclusion

On comparative evaluation of results of this in vitro study, it was concluded that ES-BCRR, Biodentine & MTA exhibited microleakage with Group II (ES-BCRR) showing the least microleakage of all. There was a statistically significant difference in the microleakage values between Group - II (ES-BCRR) & Group - I (MTA), and Group – II (ES-BCRR) & Group - III (Biodentine).There was no statistically significant differences in the microleakage values between Group - I (MTA) and Group - III (Biodentine). This study was a humble effort to evaluate the sealing ability of the newly introduced materials Endosequence Bioceramic Root Repair Material and Biodentine. Apical seal of root end filling material is the single and most important factor in achieving success in surgical Endodontics. ES-BCRR can be considered a possible alternative to the other bioactive materials as root end filling material due to its better sealing ability. However, further in vitro and in vivo investigations should be conducted to determine the suitability of ES-BCRR for clinical application.

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