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Research Article

Sealability of Bioceramic cements on root ends prepared using a Hard tissue LASER evaluated by Stereomicroscope - An *In Vitro* Study.

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Abstract

Aim: The objective of this study was to evaluate and compare the microleakage exhibited by three different root end filling materials, BioAggregate, Biodentine and MTA plus, in root ends resected and root end cavities prepared with Er:YAG hard tissue laser using dye penetration technique under stereomicroscope.

Methodology: Fifty-six freshly extracted human maxillary central incisors were selected which showed closed apices and straight canals, they were decoronated at the level of CEJ and obturated. The canal orifices were sealed with Composite material. All samples were stored at 37 ± 1°C and 100% relative humidity for 21 days. A VSP Er:YAG laser device was used to prepare root-end Class I cavities. The cavities were enlarged and deepened to 3 mm depth and 2 mm diameter with laser. The prepared root end were then sealed with bioceramic (MTA Plus, Biodentine and Bioaggregate). They were then evaluated for microleakage using dye penetration (Rhodamine B dye) and evaluated under stereomicroscope.

Results: The mean microleakage values, arranged in order, starting from the least to the maximum, are Group II MTA Plus (1.1042) < Group III < Biodentine (1.2083) < Group I Bioaggregate (2.1806).

Conclusion: The obtained results showed that dye leakage was present with all the three materials, with the highest values exhibited by Bioaggregate when compared to MTA plus and Biodentine, which showed significantly less amount of leakage.

Keywords: Surgical Endodontics; Root Resection; Root End Filling; Bioceramics; Dye Penetration; Microleakage; Er:Yag Laser

Introduction

Restorations are aimed at various teeth, mutilated teeth are conserved by crown fabrication and teeth with pulpal involvement are treated by endodontic treatment [1]. One thinks of pulpagia, toothache, as the most common symptoms of pulp pathology. Pulps suffering from irreversible pulpitis cannot be saved [2].

Chronic pulpitis is totally irreversible and the tooth must be endodontically treated. Historically, a mechanistic approach to root canal treatment was frequently adopted, but in recent years a greater awareness of the complexities of the root canal system has led to the development of newer techniques, instruments and materials.

Traditionally the 'endodontic triad' concept of cleaning, shaping and filling has been promulgated widely. However, considering that a major goal of root canal treatment is removal of microorganisms from the complex root canal system. It would therefore appear that 'shaping to facilitate cleaning and filling' might be achieved while ensuring conservation of root structure and maintaining canal shape [3].

Root canal sealers along with solid core material play a major role in achieving this fluid tight seal. Leakage is considered a common reason for the clinical failure of the endodontic therapy. Howland and Dumbsha stated 'although all root canal sealers leak to some extent, there is probably a critical level of leakage that is unacceptable for healing and therefore results in endodontic failure [4].

The three-dimensional sealing of root canal is the final phase of endodontic treatment and is essential for preventing reinfection of the canal and for preserving the health of the periapical tissues, thereby ensuring success of the root canal treatment [5]. Conventional endodontic treatment is reported to succeed in in 79% - 96% of cases. If it fails, revision of the orthograde root filling should be considered [6]. In case a non-surgical treatment fails to treat perpendicular lesions of endodontics origins or retreatment is not indicated, due to complexity of root canal systems, presence of physical barriers or presence of non-healing periapical lesion with root resorption, a surgical alternative may be considered.

Surgical endodontic intervention has emerged over the past 150 years as a significant treatment modality in the retention of sound teeth. While the evolution of this treatment modality and the refinement of its principle have had a long tumultuous history, biologically based and clinically updated directive have emerged [7]. Apicoectomy with retrograde obturation is widely applied procedure in endodontics, when all efforts for successful completion of orthograde endodontics therapy have failed [8]. The primary goal in apical resection is to achieve a hermetic sealing between the apical portion of the root canal and periapical tissues can be achieved [9].

In the late 19th century, surgical endodontic was extensively used by dentist to achieve successful and predictable prognosis in cases of failed orthograde endotherapy. Whitehouse, in the year 1884- 130 years ago chided his colleagues to minimize the extensive use of surgery and concentrate on the problem at hand, "A few moments consideration of the original cause of trouble at the apex of root will enable us to realize what is required to be accomplished in the way of unsuccessful treatment. If the original cause is admitted to be irritation from decomposing pulp, its removal will in most cases affect a cure" [7].

According to Frank., *et al.* "Surgery was used promiscuously as a connective technique following an inadequate theory." For example, gross endodontic overfill caused by combined errors of inaccurate root length measurement and apical perforation led to apical curettage and Apicoectomy to remove the over-fill [10]. Systematic reviews by Del Fabbro., *et al.* (2008) and Torabinejad., *et al.* (2009) have compared the success rates of non-surgical and surgical endodontic treatment. These results should be interpreted cautiously because they are influenced by case selection and study inclusion criteria. Surgically treated cases appear to show higher success rates after one year. However, after 2 - 4 years relative success rates appear equivalent or reversed [11].

Numerous materials have been proposed for this reason. An ideal root-end filling material should be biocompatible, antibacterial, non-toxic, non-corrosive, non-resorbable, dimensionally stable, easy to handle, unaffected by moisture, radiopaque, cost-effective, adaptable to the dentinal walls and finally able to induce regeneration of the PDL complex, specifically cementogenesis over the root-end filling itself [12].

Several materials have been suggested and clinically tried as root-end filling materials, however, each one of the materials have their own limitation. Several material have been suggested and clinically tried as root end filling materials, however, each one of the materials have their own limitations [13]. A plethora of materials have been suggested for use as root end filling materials such as polycarboxylate cements, zinc phosphate cement, zinc oxide eugenol cement, Intermediate restorative material, Cavit, Glass ionomer cement, Composite resin restorations, Gold foil and leaf, Silver point, Hydron, Diaket root canal sealer, Titanium screw and teflons, LASERS, Calcium phosphate, castor oil polymer, Super EBA etc. [6].

The Father of the family of calcium silicate cements, innovatively introduced as tooth filling material in 1995 by Torabinejad as Grey ProRoot^R MTA (by Dentsply International Inc., York, PA, USA.) composed of Grey Portland Cement containing bismuth oxide as a radiopacifiers. Calcium Silicate Cement seen to have intrinsic properties suitable for their clinical use which include as root end barriers, such as good sealing correlated to expansion, such as good sealing correlated to expansion, ability to set in presence of fluids, bioactivity, the release of ions acting as epigenetic signal and good biological properties [14]. Biodentine was introduced in 2010 by Gilus and Oliver [14]. Biodentine is another calcium silicate-based material with good handling property and relatively fast setting compared to MTA. It is used for pulp capping, repairing perforations, resorption defects and for apexification procedure. The seal and gingival fibroblasts attachment provided by Biodentine is comparable to MTA and other calcium phosphate-based cements with less cytotoxic effects compared to GIC [15]. Another recently introduced calcium silicate-based cement is Bioaggregate, which is composed of hydraulic calcium silicates, calcium phosphate, amorphous silicon oxide and tantalum oxide, contained in a crystalline mass, not separable into individual components [16]. Initial experiments have shown biocompatibility and sealing ability of bioaggregate was comparable to that of MTA [17].

For a proper root-end filling to be placed, a well-designed root end cavity is necessary. Root-end cavity can be prepared by a bur or an ultrasonic instrument. The researches have demonstrated that root-end preparations with burs are seldom straight and often obliquely placed due to difficulty in placement and head size.

Other studies have demonstrated that ultrasonic instruments create more microfractures than bur during root-end cavity preparation [18]. Lasers, if used properly with optimal setting for target tissues, yield favorable results, including a disease in dentine permeability, cavity preparations without vibrations, less crack formation on root canal walls, improved bactericidal activity and efficient removal of the smear layer and debris. Lasers have been shown to preserve the integrity of root-end cavities better than ultrasonic devices from the stand point of chipping off [19].

Thus, it has become necessary to evaluate the various different materials available for use as well as the various methods of root end preparation and device the most efficient match of the materials and methods available for application. We have used AH plus as the sealer material in this study, in lieu to its excellent properties, such as low solubility, small expansion, adhesion to dentin and its very good sealing ability AH Plus is looked as a bench mark ("Gold Standard") [20]. AH Plus has the following characteristics: long term sealing, great dimensional stability, bonding properties, high radiopacity, biocompatibility and antimicrobial activity [21].

The sealablity of root-end filling materials have been assessed by different methods such as dye/ink (methylene blue, eosin, basic fuschin, silver nitrate and gold palladium) or electrochemical methods fluid filtration technique, radioisotopes tracing and evaluation of marginal adaptation by scanning electron microscope. Among the aforementioned methods, dye penetration studies are the most commonly used technique for microleakage assessment of root-end filling materials [9,22]. Rhodamine B dye has been used to evaluate the sealing capacity for the dye penetration test.

Method of collection of Data

Fifty-six freshly extracted human maxillary central incisors were collected from the Department of Oral and maxillofacial surgery, Rajasthan Dental College and hospital and stored in normal saline until use. Criteria for teeth selection included: a single root canal without curvature; no visible root caries, fracture or cracks on examination; no signs of internal or external resorption or calcification; and, completely formed apex.

Specimen Preparation: Fifty-six freshly extracted human maxillary central incisors were selected which showed closed apices and straight canals, which were stored in normal saline until use. The teeth were cleaned ultrasonically and later decoronated at the level of CEJ with a diamond cutting disc mounted on a micromotor. Canal orifices were located and pulp extirpation was done with the help of K-files and canal patency was confirmed. 15 No. K-file was used to determine the working length with each root specimen. Root canals were prepared using step-back technique until reaching a master apical file size 50No. K-file. The canals were irrigated between instruments with 2 ml of 5% sodium hypochlorite (NaOCl). Finally, the root canal was irrigated with 5 ml of 5% NaOCl. Canals were obturated with gutta-percha and AH plus sealer (Dentsply Maillefer, Ballaigeus, Swaziland) using lateral compaction technique. Excess gutta-percha was removed with a heat-carrier and remaining gutta-percha was vertically condensed at the canal orifices. Radiographs were taken to confirm the quality of obturation and the canal orifices were sealed with Composite material. All samples were stored at 37 ± 1°C and 100% relative humidity for 21 days.

Root-end Cavity preparation: A VSP Er:YAG laser device (Fidelis Plus, Photona, Ljubljana, Slovenia) was used to prepare root-end cavities (Parameters: 4w, 45% Water, 55% air). The Er:YAG laser emitted pulsed infrared electromagnetic radiation at a wavelength of 2.94 μm through a system of seven mirrors in a fully operating mobile arm. An RO04 handpiece with a focus of 940 μm from the same manufacturer was used for preparation. The experiment was carried out under constant water cooling. The cavities were enlarged and deepened to 3 mm depth and 2 mm diameter.

The teeth were randomly divided into 3 groups of 18 specimens each:

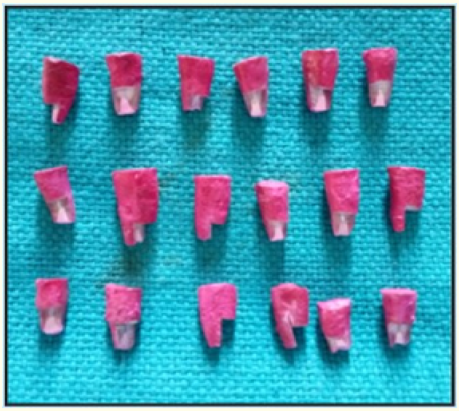
1. **Group I:** BioAggregate
2. **Group II:** Mineral Trioxide Aggregate (MTA Plus)
3. **Group III:** Biodentine

These materials were manipulated according to the manufacturer's instructions and the cavities were filled using a MTA carrier (GDC). Two coats of nail varnish were applied to the external surface of each root. Teeth were removed from saline and placed in small plastic containers. The roots were then totally immersed in a solution of Rhodamine B dye for 24h. After removal from the dye, teeth were rinsed under tap water for 30 minutes and using a diamond disc, each root was longitudinally sectioned into two halves.

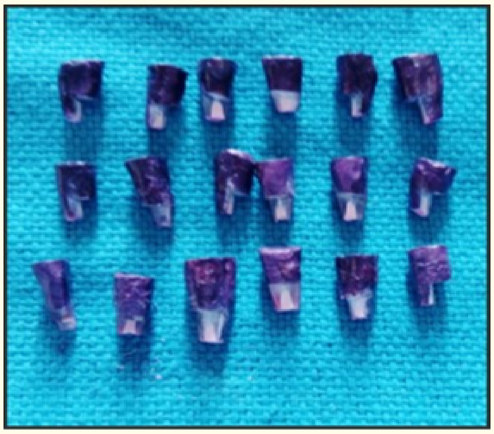
Figure 1: Root End prepared with the help of Er: YAG laser along with its radiograph.

Group	**Material used**	**Composition**		**Manufacturer**
		Powder	**Liquid**	
Group - I	BioAggregate	Tricalcium silicate Dicalcium silicate Tantalum pentoxide Amorphous silicon -oxide Calcium phosphate -monobasic	Ionised water	Innovative BioCeramix Inc., Vancouver, Canada
Group - II	MTA Plus	Tricalcium silicate Bismuth oxide Dicalcium silicate Tricalcium aluminate Calcium sulfate -dehydrate	Special MTA plus Gel	Prevest Denpro Limited, Avalon Biomed Inc,. USA
Group -III	Biodentine	Tricalcium silicate Dicalcium silicate Calcium carbonate	Aqueous calcium chloride solution with excipients	Septodont, France

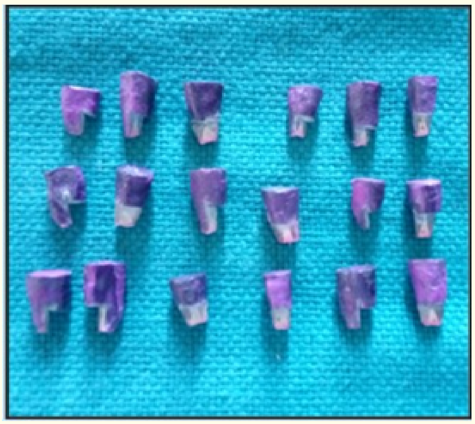
Table



1. Samples with Bioaggregate Root End Filling



2. Samples with Biodentine Root End Filling



3. Samples with MTA Plus Root End Filling

Figure 2

Citation: Amit Patodiya., *et al.* "Sealability of Bioceramic cements on root ends prepared using a Hard tissue LASER evaluated by Stereomicroscope - An *In Vitro* Study". *Acta Scientific Dental Sciences* 2.8 (2018): 09-18.

Each specimen was then examined as to the adaptation of the root end filling material to the cavity walls and the extent of dye penetration using stereomicroscope (30X) and microleakage was evaluated in millimeters.

Results: The obtained results showed that dye leakage was present with all the three materials, with the highest values exhibited by Bioaggregate when compared to MTA plus and Biodentine, which showed significantly less amount of leakage.

Results revealed statistically significant difference between Bioaggregate and MTA Plus ($p < 0.05$) and between Bioaggregate and Biodentine ($p < 0.05$). However, no statistically significant difference was observed between Biodentine and MTA Plus ($p < 0.05$).

In the present study, the mean microleakage values of Group I (Bioaggregate) is 2.1806 with a standard deviation of 0.400 mm, Group II (MTA Plus) is 1.1042 mm with a standard deviation of 0.351mm and Group III (Biodentin) was 1.2083 mm with a standard deviation of 0.340 mm.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
BioAggregate	18	2.1806	.40042	.09438	1.9814	2.3797	1.38	2.88
MTA plus	18	1.1042	.35160	.08287	.9293	1.2790	.50	1.75
Biodentine	18	1.2083	.34031	.08021	1.0391	1.3776	.50	1.88
Total	54	1.4977	.60632	.08251	1.3322	1.6632	.50	2.88

Table 1: Descriptives.

The mean microleakage values, arranged in order, starting from the least to the maximum, are Group II MTA Plus (1.1042) < Group III < Biodentine (1.2083) < Group I Bioaggregate (2.1806).

Comparison of all the three study materials (in millimeter)

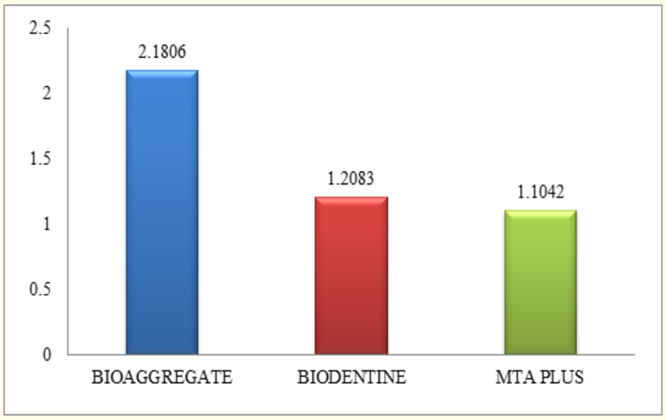


Figure 3

It is thus observed that MTA Plus and Biodentine both possess better sealing capacity when compared with Bioaggregate with the maximum sealing capacity was demonstrated by MTA Plus, closely followed by Biodentine. However, the least value for sealing capacity was presented by Bioaggregate.

ANOVA was applied to test for any possibility of equality of mean among the categories was present. Results indicated that High Significant differences were observed between group I to Group II and Group I to Group III, meanwhile no significant difference was present between the Group II and Group III. The statistical significance is considered if $p > 0.05$.

Independent sample t-test was used for comparison between the samples of each group. There was no significant difference in the microleakage values between the samples of Group I ($P = 0.094$), Group II ($P = 0.082$) and Group III ($P = 0.80$).

All the data are entered into Microsoft excel sheet and subjected to statistical analysis. Test of normality was done using Kolmogorov Smirnov test and Shapiro-Wilk tests was used. Mean and standard deviation was done for descriptive statistical analysis. Independent sample t test was done to compare the two groups. For comparison of all the three-study materials analysis of variance test was used. Bonferroni post hoc test was used for multiple comparison. P value < 0.05 was used for statistical significance analysis SPSS -17 version used for statistical analysis.

Discussion: Microorganisms play a very important role in pulpo-periapical diseases and thus the primary aim of conducting a root canal treatment is the eradication of the pathological microflora in the canals as well as providing a hermetic seal to prevent the movement of microorganisms or the microbial toxins to and from the canal system and periapical tissues. The prime requisite for a successful treatment and predictable prognosis is the apical seal which in turn depends on proper instrumentation and cleaning of the coot canal system.

However, several factors inherent to the endodontic procedures, such as perforations, instruments blockage, calcifications and anatomic anomalies can lead to treatment failures. In some cases, conventional endodontic treatment is not sufficient to solve the problem and surgical endodontics intervention is required. This involves the exposure of the involved root apex, resection of its apical end, root-end Class 1 cavity preparation and insertion of an appropriate root-end filling material [23].

Apical ramifications and lateral canals are very common near the root tip. Resection at the depth of 3 mm reduces the apical ramifications by 98% and lateral canals by 93%. During preparations of root end cavity, good visualization and easy access are main criteria for choosing 0', 30' or 45' resection angles. 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. Gagliani., *et al.* and Gilheany., *et al.* in their studies stated that the microleakage increased significantly with increased angulations of the resected root-end [23].

Widely, Apicoectomy is performed mainly using a rotary cutting device in clinical settings, but there is concern that the prognosis may be affected by frictional heat, cracks formation and excessive dentinal removal causing damage to the tooth structure. The advantage of using ultrasonics for root end preparation is their smaller dimensions of the cavity prepared and improved access to the resected root-end cavity. However, its use has been shown to produce cracks on root canal walls [19].

Therefore, in this study LightWalker Hard and Soft Tissue Laser device made by Fotona, USA was used. It is a Er:YAG laser with a pulse rate of 20 Hz, with output of 6 W at MSP mode with energy levels at 300mJ, used with a non-contacting tip ($R0_2$) [24].

To make sure that the root end resection was done at proper length of 3 mm, each of the sample was measured and marking made at 1.5mm and 3mm from the apex. The root end cavity preparation was also done at the same setting with Er:YAG laser and non-contacting tip ($R0_2$) to achieve Class I retrograde cavities of dimension 3 mm deep and 2 mm wide which were confirmed with the help of Hu-Friedy periodontal probe.

The presence of smear layer on the cut dentin surface when prepared with bur or ultrasonics can lead to gap formation as well as sequestration of bacteria and harmful substances in the fragments created by mechanical cutting is disadvantageous and is indicated for removal. The presence of smear layer has also shown to reduce the sealant adhesion to dentin and may thus affect prognosis and it thus become necessary for it removal.

However when the dentin surface that was prepared with Er:YAG laser was analysed, no marked evaporation, fissures and carbonization was observed and thus the absence of a smear layer was noted. The principle of hard tissue cutting using Er:YAG laser is that instead of dissolving dentin, a few water molecules in a micro-area irradiated with laser vaporize by rapidly absorbing energy and this force increases the internal pressure to cause a microexplosion, thus physically destroying hard tissue. During the series of cutting, the smear layer is also washed away by water irrigation. Furthermore, the 2.94 um oscillatory wavelength of the Er:YAG laser resembles the maximum absorption band of water and the heat generated during laser application is lower when compared to the Nd:YAG laser or CO_2 laser [25].

On evaluation of the cavities prepared by the laser it was noticed that despite being a pulsed laser, the walls were comparatively smooth and rounded, however, with an apically divergent wall, which may have contributed to the increased dye penetration, as well as the reduced retentive capacity of the cavities towards the root end fillings, particularly Bioaggregate.

On the positive side of the use of Er:YAG laser was the efficiency with which cavities were prepared [23], as well as the ease during both Apicoectomy and cavity preparation with an approximate time of 2 minutes for resection and about 3 minutes for root end preparation with time also included for evaluation of the depth and with of the cavity. The time required on an overall procedure was close to 4 minutes per tooth as the efficiency of the operator increased over time.

Dye penetration, which is often used for leakage studies because dyes are relatively easy to be stored, applied and have their penetration assessed quantitatively. Rhodamine B an organic dye compounded by a red-violet powder is classified as a xanthenic dye. It presents greater diffusion on human dentin than methylene blue. According to Franci, the molecules of rhodamine B are nanometric and are optimal to simulate enzymes and toxins of leakage resulting from bacterial metabolism. Rhodamine B dye has been the most preferred dye for confocal microscopy due to its fluorescence [23].

Post., *et al.* compared the degree of dye penetration in MTA and amalgam. It was concluded that the penetration in MTA is lesser and no connection was established between the apical preparation and the degree of dye penetration. Kuzmanova and Nikiforova have done a measurement of microleakage in four different materials for retrograde filling - gray MTA, Adhesor, Astralloy, AdSeal at an angle of 45 degrees and 3 mm apical resection using a traditional technique. They established that the lowest microleakage was observed for gray MTA - from 0.34 to0.67 mm, while in amalgam the microleakage was most expressed - from 2.8 to 0.44 mm [26].

Biodentine is actually formulated using the MTA-based cement technology and the improvement of some properties of these types of cements, such as physical qualities and handling. The Biodentine powder also has inclusions of calcium carbonate which were relatively large compared to cement particles. There are hydration products around the circumference of the calcium carbonate particles. The authors added that calcium carbonate acts as a nucleation site, enhancing the microstructure [27].

Since the basic components of Biodentine are similar to MTA, these materials are expected to have similar properties and effects. These results were in comparison with the studies done by Ozbay., *et al.* and Saravanapriyan., *et al.* They compared the sealing ability of Biodentine with MTA and concluded that MTA has better sealing ability than Biodentine.

Though, there was no statistically significant difference between Biodentine and MTA, the minor variation in the lower microleakage values of MTA may be attributed to its superior marginal sealing ability resulting from its hydrophilic properties and formations of an interfacial layer between the material and dentin. The interfacial layer reduces the risk of marginal percolation and gives promising long-term clinical success.

Kubo., *et al.* found that the further hydration of MTA powder by moisture can result in an increase in the compressive strength and decrease leakage. Saker., *et al.* demonstrated that MTA has the ability to precipitate hydroxyapatite crystals in the presence of fluid which may be relevant in minimizing leakage thereafter. In our study, similar results were observed, with no statistically significant difference between MTA Plus (1.1042 mm) and Biodentine (1.2083 mm).

A study performed by Torabinejad M., *et al.* [28] did not reveal any significant solubility of MTA whereas; Fridland M and Rosado R have reported the significant increase in solubility and porosity of ProRoot MTA with the increase in water to powder ratio [29]. De Souza ET., *et al.* conducted a study on porosity and compared Biodentine with IRoot BP, Ceramicrete and ProRoot MTA using micro-CT characterization. They observed that no significant differences were found in porosity between the new calcium silicate containing repair cements and MTA [30]. Due to low water content in the mixing stage Biodentine exhibits lower porosity than MTA.

Biodentine is found to be associated with high pH[2] and releases calcium and silicon ions which stimulates mineralization and create "mineral infiltration zone" along dentin-cement interface imparting a better seal. Caron G., *et al.* have found that Biodentine exhibits superior sealing properties than MTA [31]. While Torabinejad M reviewed a comprehensive literature to investigate studies regarding the leakage of MTA and concluded that MTA has good sealing ability and it seals well [32]. Ravichandra PV., *et al.* evaluated that Biodentine provide better adaptation and seal than commonly used root-end filling material [33]. However, Ozbay G., *et al.* observed less microleakage with MTA then Biodentine when analysed by fluid filteration method [34].

Micromechanical adhesion of Biodentine enabled excellent adaptability of Biodentine crystals to the underlying dentin [13].

Soundappan S., *et al.* conducted invitro study to compare the marginal adaptation of Biodentine with MTA and Intermediate Restorative Material (IRM) using scanning electron microscope and concluded that both MTA and IRM were significantly superior to Biodentine in terms of marginal adaptation when used as a root end filling material [35].

Hydrated mineral trioxide aggregate (MTA) leaches calcium hydroxide in solution. The leaching pattern of calcium hydroxide varies when the material is in contact with water or simulated body fluid with more calcium ions released in physiological solution. Calcium silicate-based cements deplete the free phosphorus present in solution in simulated body fluids. MTA in contact with tissue fluids results in the deposition of hydroxyl apatite. An amorphous calcium phosphate phase is initially formed which is later transformed to an apatite phase, the latter consisting of poorly crystalline B-type carbonated apatite crystallites [24].

The better performance of MTA Plus could be attributed to the finer particle size and the presence of an anti-washout gel which drastically increase the anti-washout resistance of MTA Plus. Camilleri., et al. determined that the crystalline particles in MTA Plus were smaller (50% of the particles finer than the 1 μm) than those present in ProRoot MTA although the chemical composition were found to be similar. Smaller particle size is important for physical properties as it will increase the surface available for hydration and cause greater early strength as well as ease of handling.

These findings agree with the results of previous dye leakage studies which have been performed on MTA using various different types of dyes [36]. The better performance of MTA may be due to its superior marginal sealing ability resulting from its hydrophilic properties [37] and formation of an interfacial layer between the material and dentin [38]. It was found that the further hydration of MTA powder by moisture can result in an increase in the compressive strength and decrease leakage [39]. In addition, it was also demonstrated that MTA has the ability to precipitate hydroxyapatite crystals in the presence of a fluid which may be relevant in minimizing leakage thereafter [40].

Camilleri and Farmosa., *et al.* in their study observed that Bismuth was present in the hydrated MTA plus and was incorporated in the calcium silicate hydrate structure. This finding is similar to reports of ProRoot MTA where the bismuth oxide phase was not acting as a filler but was involved in the hydration reaction of MTA [15]. In MTA, only 8% of unbound bismuth oxide was detected in the hydrated form from the original 21% present in the unhydrated material. The losses can be attributed to the binding of bismuth phase to calcium silicate hydrate and leaching of bismuth in solution [15].

As Bioaggregate is a relatively new material, there are only few studies available about this material. BioAggregate Root Canal Repair Filling Material reacts chemically with BioAggregate Liquid or water and that specific intermediate products of this reaction i.e. calcium hydroxide, a common material used in dental procedures, is present only while the product is setting or curing [41].

According to our present study, Bioaggregate (2.1806 mm) showed the maximum amount of microleakage when compared to MTA (1.1042 mm) and Biodentine (1.2083 mm), though they have nearly the same components. Thus, Bioaggregate has shown to be the least capable of producing a proper seal exhibiting maximum microleakage values with statistically significant differences when compared to either MTA Plus or Biodentine.

However, on the contrary, studies have shown that the sealing capability of Bioaggregate is combined with excellent biocompatibility and significant stimulation of periodontal regeneration [42]. In one study conducted by Al Sayed., *et al*. Bioaggregate showed the least amount of microleakage; surprisingly, even when compared to MTA [7].

Microleakage was found to be significantly less in Bioaggregate (0.22 mm) when compared to Biodentine (0.37 mm) ($p < 0.001$) and with MTA (0.58 mm) ($p < 0.001$) [43].

In another study conducted to evaluate the antimicrobial effectiveness of bioaggregate and mineral trioxide aggregate against *Enterococcus faecalis in vitro* revealed that they were equally effective in bacterial elimination and caused a significant decrease in bacterial viability within 6 minutes [44].

The seal associated with this bioaggregate could be explained by the following

(1) It has nano-sized particles that achieve excellent adhesion to the dentinal walls of the root canal.

(2) It is hydrophilic in nature demonstrating setting expansion.

The presence of a gel-like calcium silicate hydrate as the main structural component in both the MTA and Bioaggregate, provides strength, hardness and sealing properties to the set material [45].

Thus, within the limitations of our study, it was concluded that of the three materials evaluated, all the three materials exhibited microleakage. Of these, MTA Plus and Biodentine exhibited less microleakage when compared to the newly introduced Bioaggregate, with MTA Plus showing the least values of microleakage. However, the difference between the values for MTA Plus and Biodentine was not statistically significant, while the difference in values presented by Bioaggregate were of statistical significance, which were as high at approximately twice the values of MTA Plus and Biodentine [46].

Conclusion

From the following study the following conclusion can be derived

- MTA Plus is a better material as root end filling material to prevent microleakage.
- Though MTA plus is better than Biodentine in respect to microleakage, no statistically significant difference is present between the two.
- Maximum amount of dye penetration was exhibited by Bioaggregate root end filling material.
- Further *in vivo* studies are needed to correlate with findings of the present study.

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