

Comparison of Marginal Adaptation of Endodontic Biomaterials as Apical Plugs in Simulated Open Apex Cases- An Invitro Study

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Abstract

Introduction: This study was done to compare the marginal adaptation of Bio dentine, Endocem MTA and nano MTA apical plugs to the root canal dentin in simulated open apex cases.

Materials and Methods: Thirty mature single root ed teeth were collected and crowns of the teeth were sectioned to standardize length of 15 ± 1 mm. Working length was established and apical instrumentation of roots were done to a size of 40 K file and canals were flared up to #80 with the step back technique. Apical foramen were enlarged using Peeso drills #1 through #4, to simulate an open apex. Specimens were assigned to 3 groups: group A (Bio dentine, n=10), group B (Endocem MTA, n=10) and group C (Nano MTA, n=10).

Bio dentine™, Endocem MTA and Nano MTA apical plugs were prepared according to the Manu facturer’s instructions and condensed up to the apical end of the roots to form apical plugs of 3mm. Slices of sponge

were soaked in PBS solution and teeth were placed in these slices to simulate periapical tissue and stored in an incubator.

After 24 hours, the specimens were sectioned and viewed under SEM. The images were used to evaluate the marginal adaptation between the endodontic biomaterials and root canal wall.

Results: Nano MTA showed better marginal adaptation to the root canal dentin which was followed by the Endocem MTA group and the least marginal adaptation was shown by the Bio dentin group.^[28]

Conclusion: The most marginal adaptation of the apical plugs with the root canal wall was seen in the case of Nano MTA, followed by Endocem MTA groups and the least was seen in the Bio dentine groups.

However, further research is required for properties of Nano MTA and Endocem MTA to assess its use as an ideal root-end filling material apart from its marginal adaptability.

Keywords: Open apex, marginal adaptation, Bio dentine, Endocem MTA, Nano MTA

Introduction

The science of endodontics has been always looking for better ways for the treatment of immature necrotic teeth.^[6] Trauma or caries might result in pulp necrosis in young permanent teeth. Pulp necrosis in immature permanent teeth will cause cessation of root closure and root maturation as completion of root development and closure of the root apex takes up to 3 years after eruption of the tooth. Thus, canal walls become thin and fragile and apex of tooth remains open.

Under these circumstances, root canal instrumentation will not enable the achievement of an adequate apical stop.^[8] Endodontic treatment of immature permanent teeth with open apices involves inducing apical closure by apexification procedures. This technique involves removal of the necrotic tissue, debridement of the canal and placement of an antimicrobial material for induction of a calcific apical barrier.

Many materials have been used for apexification ranging from antiseptic pastes to calcium hydroxide to the more recent use of bio ceramics.^[1] Bio dentine is a new calcium silicate-based cement with dentin-like mechanical properties.

It has a good sealing ability and has shown favourable biologic response. Bio dentine has endodontic indications similar to MTA and it can be used as a retrograde apical filling.^[8] Endocem MTA possess a very similar chemical composition to that of MTA, but present ease of manipulation and the ability to set quickly because of the pozzolanic particles.

A nano modified version of MTA known as “Nano MTA” sets faster with acceptable resistance to acidic environments. Reducing the particle size plays an important role in shortening the setting time and

increasing the micro hardness even at low pH.^[3]

These bioactive materials improve the sealing ability of the apical barrier when compared to older materials. However, clinical failures are still a recurrent problem, mostly due to micro leakage.^[1] Studies indicate that 88% of endodontic treatment failures are due to apical microleakage.^[6]

Evaluation of marginal adaptation of root-end filling materials by means of scanning electron microscopy (SEM) can provide information regarding their sealing capacity.^[39] Hence, this study was designed to compare the marginal adaptation of Bio dentine, Endocem MTA and Nano MTA apical plugs to the root canal dentin in simulated open apex cases.^[1]

Materials and methods: Thirty a traumatically extracted mature single rooted teeth with straight intact root canal were collected, stored and surfaced adhering to infection control protocols in the laboratory as prescribed by Center for Disease Control Global.

Inclusion criteria for the teeth were as follows

A minimum tooth length of 15 ± 1 mm and radiographically verified straight root canal. Exclusion criteria were as follows: teeth presenting a previous restorative treatment (i.e. amalgams, composites, or fixed prosthetics), radicular caries, severe dilacerations, hyper cementosis, previous root canal treatment, root canal obliteration, isthmuses, calcifications, or presence of bifurcations or lateral canals. After final evaluation, 30 teeth were selected.

Specimen preparation

For disinfection, the specimens were stored in 5.25% sodium hypochlorite for an hour and then placed in normal saline. The crowns of the teeth were removed by sectioning with a diamond disc to standardize length of 15 ± 1 mm at 250 RPM under copious water irrigation (Iso Met 1000 Precision Cutter, Buehler Co. Illinois,

USA). Real root canal lengths were determined by manually inserting #15 K files into the canals, until the instrument tips were visible at the apical foramen. Working length was established 1.0mm shorter than real root canal length.

Apical instrumentation of roots was carried out with stainless steel files to a size of 40 K file as the master apical file. Canals were then flared up to #80 with the step back technique. Apical foramen were enlarged by using Peeso drills sequence #1 through #4, (Dentsply-Maillefer®, Ballaigues, Switzerland) to simulate an open apex, the internal diameter was then standardized to 1.3mm.

Canals were then irrigated with 1.0mL of 2.5% sodium hypochlorite. After instrumentation was completed, 3.0mL of 17% EDTA was introduced and allowed to remain in the canals for 3 minutes. Next, a final flush with 1.0 mL of 2.5% sodium hypochlorite followed by 5.0mL of normal saline was done. Specimens were randomly assigned to 3 groups as follows: group A (Bio dentine, n=10), group B (Endocem MTA, n=10) and group C (nano MTA, n=10).

GROUP 1- Bio dentine™

Bio dentine™ (Septodont Co., St. Maur-Des-Fosses, France) was prepared according to the Manufacturer's instructions, and carried and condensed up to the apical end with aid of calibrated endodontic plugger RCP 9/11(Hu-Friedy®, Illinois, USA) to form apical plugs of 3mm. The excess material was removed using #80 paper points (Hygienic Corp. Ohio, USA).

Slices of sponge were soaked in PBS solution and teeth were placed in these slices to simulate periapical tissue to avoid dehydration of samples. All specimens were prepared within a 5 min period, and then stored in plastic vials at 37°C in 95% humidity in an incubator for 24 hours.

Group 2: Endo cem MTA

Endocem MTA (Maruchi, Wonju, Korea) apical plugs were created identical to group 1.

Group 3: Nano MTA

Nano MTA (UNESP, Ilha Solteira, Brazil) apical plugs were prepared the same way as group 1 and 2.

After 24 hours, the specimens were sectioned and prepared for scanning electron microscopy. SEM images were used to evaluate the marginal adaptation between the endodontic biomaterials and root canal wall.

Marginal adaptation analysis

Samples were sectioned using diamond discs and the specimens were prepared for scanning electron microscope (SEM) (Jeol Ltd, Tokyo, Japan) analysis and were mounted on specific metallic stubs to prevent their movement and to allow the evaluation to be made parallel to the long axis of the foramen.

Specific parameters of 5 kV and 100x, 250x and 750x were used and a single trained examiner performed the blind evaluations of SEM images and photomicrographs were taken to evaluate marginal adaptation.

Statistical analysis

Inferential statistics like Kruskal-wallis was applied to check the statistical difference of marginal adaptation among the groups and post-hoc mann Whitney was done for pair-wise comparison.

Results

Thirty experimental specimens were evaluated for marginal adaptation using the scanning electron microscopy. Representative SEM (100x, 250x and 750 x) analysis are shown in Figures [1-3] for Bio dentine™, Endocem MTA and Nano MTA apical plugs respectively. All specimens showed acceptable and homogeneous marginal adaptation between the materials and the dental structure. [Figures 1-3]. Bio dentine [FIGURE 1] showed in all samples a

heterogenous surface full of crystal line structures with more width at the dentin – Bio dentin interphase.[1]

Endocem MTA [FIGURE 2] showed better homogeneity than Bio dentine in the biomaterial core and lesser gap at the dentin – Endocem interphase in comparison to the dentin – Bio dentin interphase. Nano MTA [FIGURE 3] showed the best homogeneity within the biomaterial core, and very less abrupt changes in the dentin-Nano MTA interphase among all the three groups.

Table 1 illustrates the comparison of mean Marginal Adaptation gap values between 3 groups. The test results demonstrate that the mean Marginal Adaptation gap values for Bio dentin group was 26.490 ± 12.728 , for Endocem MTA group was 6.125 ± 2.080 and for Nano MTA group was 4.198 ± 0.929 .

This mean difference in the Marginal Adaptation gap values between 3 groups was statistically significant at $P < 0.001$ [Refer Graph no. 1]

Table no.2 illustrates the multiple comparison of mean differences in Marginal Adaptation gap values between 3 groups. The test results showed that the Nano MTA group showed significantly less Marginal Adaptation gap values as compared to Bio dentin and Endocem MTA group at $P < 0.001$ & $P = 0.03$ respectively.

This was then followed next by, Endocem MTA group showing significantly lesser mean marginal adaptation gap as compared to the Bio dentin group at $P < 0.001$.

This infers that Nano MTA showed significantly least mean marginal adaptation gap as compared to other groups, which was followed by Endocem MTA group and highest with Bio dentin group. [Refer Graphs no. 2 - 5].

Discussion

In the present study, the marginal adaptation of apical plugs containing Bio dentine, Endocem MTA, and Nano MTA to the root canal wall was evaluated in vitro using

SEM observations. Success of endodontic treatment depends on the coronal and apical sealing of the root canal. Importance of apical plugs in endodontic sealing is inevitable. ^[40] Goal of apical plug is to provide a tight seal against microleakage ^[6].

Microleakage is one of the most important reasons for the failure of endodontic treatment. Studies indicate that 88% of endodontic treatment failures are due to apical microleakage.

Also, greater microleakage is observed in teeth with larger apical foramen diameters.^[6] As characteristic signs of endodontic treatment failure due to micro leakage, apical periodontitis and post-treatment symptoms can occur, indicating the need for endodontic retreatment or surgical intervention which can be time consuming and expensive.

Adequate apical sealing prevents micro-organisms and endo toxins from reaching apical and periapical tissues. ^[40] There is a correlation between microleakage and marginal adaptation that is shown in studies by Shani et al. and Torabinejad et al.^[5] Also researches have confirmed a direct link between sealing ability and marginal adaptation of the root end filling materials.

Treatment outcome is negatively affected by material's failure in marginal adaptation to the root canal dentin and spaces in the interface between the material and the dentin walls.

Investigating the marginal adaptation in open apex cases provides an indirect evaluation of sealing ability of materials used in the root canal obturation where appropriate adaptation of the master gutta-percha cone is impossible. Apexification is a method to induce a calcified barrier in a root with open apex or the continued apical development of an incomplete root in teeth with necrotic pulp. This technique involves removal of the necrotic tissue, debridement of the canal

and placement of an antimicrobial material such as calcium hydroxide for induction of a calcific apical barrier.^[6]

An ideal root-end filling material should be able to seal the root canal system and finally be able to induce regeneration of the PDL complex, specifically cemenogenesis over the root -end filling itself. ^[28]

Many clinicians prefer biomaterials, like calcium hydroxide, to enhance the gradual biological sealing of the open apex, however, several factors such as inter-appointment contamination, formation of a porous barrier, multiple-visit treatments and control appointments are still remarkable concerns and many patients may gradually abandon the treatment.

Also, the use of temporary restorative materials between multiple appointments provides no certainty on their mechanical and sealing properties. These factors may converge to balance the equation towards failure and finally all efforts could be worth less.^[1] To avoid these problems, it is recommended to perform one-visit apexification procedures or usage of apexification plugs.^[28]

Morse et al. defined one-visit apexification as the non-surgical condensation of a biocompatible material into the apical end of the root canal. The rationale is to establish an apical stop that would enable the root canal to be filled immediately. There is no attempt at root end closure, rather an artificial apical stop is created.

The interfacial strength between the material and dentin through frictional retention or micro mechanical adhesion resist dislodgement of the material and maintain the integrity of the interface.^[4]

The selection of biomaterials for apexification treatments is as complex as the procedure itself.^[1] The proper material selection with high sealing ability is necessary for apical plug creation.^[5] A number of

materials have been proposed for this purpose including tricalcium phosphate, calcium hydroxide, freeze dried bone, freeze-dried dentin etc. Recently there have been a number of reports describing the use of MTA in one-visit apexification.^[31] MTA has high sealing ability, biocompatibility and capability to stimulate osteoblasts.^[41] But MTA has been demonstrated to have some disadvantages including a long setting time, high cost, and potential for discoloration. It does not have good handling characteristics and its antibacterial properties are unpredictable.^[8] This has led to the search for other newer materials.^[41] Hence, newer materials like Bio dentine, Endocem MTA and Nano MTA could be considered to be used in one-visit apexification or as apical plugs.

Bio dentine is a calcium silicate-based restorative cement with dentin-like mechanical properties which consists of powder and liquid. The powder mainly contains tricalcium and dicalcium silicate (3CaO SiO_2 and 2CaO SiO_2) as well as calcium carbonate (CaCO_3) and zirconium oxide.

The liquid consists of calcium chloride ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) solution with an admixture of poly carboxylate. Bio dentine has a good sealing ability, good mechanical properties and has shown favourable biologic response as a pulp capping agent. Its setting time is 12 minutes and does not cause discoloration. Bio dentine has endodontic indications similar to MTA and hence it can be used as apical filling for one visit apexification treatment in teeth with open apices.^[8]

Endocem MTA (Maruchi, Wonju, Korea), can also be considered to be used as apical filling for one visit apexification treatment which has a rapid setting time (4 minutes) and good manipulation properties and main a chemical composition similar to that of MTA. The ability of Endocem MTA to set quickly is because of the

small pozzolanic particles that brings to the composition.

Pozzolan is a siliceous material, when in finely divided form, increases the area of surface contact with the mixing liquid and thus the reactivity of calcium silicate particles with water increases, forming compounds with cementitious properties (i.e, calcium hydroxide and calcium silicate hydrate phases). Then, calcium hydroxide reacts with oxides of the pozzolan in a process known as pozzolanic reaction that progresses similar to an acid-base reaction. During this reaction, additional calcium silicate hydrate and hydrate reaction products are formed, which are stable crystals that enhance the strength of the cements. Endocem MTA also has similar bio compatibility and osteogenicity / Odontogenicity comparable with MTA.

Moreover, it showed higher washout resistance under the use of several root canal irrigants and antibacterial activity against *Enterococcus faecalis*. Also it showed better push-out bond strengths compared to MTA that probably due to release of significantly more calcium and apatite like crystals. ^[4]

Nano MTA is calcium silicate-based cement composed of nano-sized particles ranging between 40 and 100 nm that provides a surface area about 4 times higher than that of MTA. An increase in the surface area of the material obtained by nano-sizing the powder particles would promote the rise in pH, increase in the calcium ion release in solution, enhances the hydration phase of cement particles and better interlocking of powder particles to create a cement structure with better integrity. It is seen that in mixing and working phase, as more particles are involved in reaction with the hydration phase, a less porous set material is formed (3). Komabayashi and Spang berg showed that MTA's particle size has a great impact on the extent of particles penetrating the dentinal tubules (Komabayeshi T.et al

2008).

The initial setting time of Nano MTA is approximately 6 min. The difference in initial setting time can be due to the increased surface area of Nano MTA than MTA thereby, Nano MTA reacts more rapidly with water and prevents washout of the cement plug before final setting. Reducing the size of particles and their uniform distribution will better accommodate and fill small gap spaces within the product and is reported to play an important role in shortening the setting time and increasing the micro hardness even at low ph.

Also, Nano MTA exhibits higher compressive strength in all pH conditions and can be advocated as an excellent root end filling material, especially when the applied material might be exposed to acidic environments. ^[3]

Thus, in the present study, the endodontic biomaterials namely Bio dentine, Endocem MTA and Nano MTA were used as apical plugs. For this purpose, the tooth specimen was standardized regarding the root canal length and the diameter of apical opening.

For the creation of open apex conditions, in different studies, Peeso Reamer and sulfuric acid have been used. Since, root resorption created by sulfuric acid caused some irregularity in canal walls and may have influence on the gap diameter between the plug and the wall, Peeso Reamer was used in this study ^[5].

Marginal adaptation indirectly reflects the sealing capacity of a root-end filling material; therefore, it has been considered as an important characteristic.

Studies have shown that the smear layer has an adverse effect on marginal adaptation of endodontic materials. Marginal adaptation is significantly increased when 17% EDTA was used. Hence, in order to remove the smear layer and increase marginal adaptation, 17% EDTA was used as a chelating agent in the present study ^[5].

The manufacturer of MTA recommends a 3 to 5 mm

thickness of MTA be placed at the apex for the apexification procedure.

The results of the earlier studies have led to a conclusion that both 1- and 2-mm apical plugs of Bio dentine and MTA might be ineffective against apical leakage. However, 3- and 4-mm apical plugs of MTA gave satisfactory results.

The amount of apical micro leakage was significantly lower for 3- and 4-mm apical plugs of Bio dentine and MTA. Hence, an apical plug of 3mm of Bio dentine, Endocem MTA and Nano MTA was used in the present study for one visit apexification.^[8]

The seal ability of root-end filling materials has been assessed by different methods such as dye/ ink (methylene blue dye, India ink, fluorescent and reactive blue dyes, eosin, basic fuchsin, silver nitrate and gold-palladium) or bacterial/ endo toxin penetration, electro chemical methods, fluid filtration technique, radio isotope tracing, and evaluation of marginal adaptation by scanning electron microscopy.

In the present study, scanning electron microscopy was used to evaluate marginal adaptation of the apical plugs with the root canal wall. SEM has some advantages in comparison with optical microscopes such as higher resolution. On the other hand, this method is more common, affordable and more accessible compared to micro-computed Tomography (micro-CT) method.^[5] In addition, the marginal adaptation can be measured quantitatively without destroying the samples.^[6]

The observations were done to ensure the measurement of width of the gap between the apical plug and the canal wall. The surface of each sample was viewed under SEM at 5 kV at 100X, 250X and 750 X magnifications and width of the gap between the plug and the canal wall was recorded for each sample.^[5] [FIGURES 1-3]

All the samples [FIGURES 1-3] when viewed under

SEM showed a good adaptation of the apical plug materials to the root canal wall, and well-defined microstructures within all the three bio material cores. Bio dentine [FIGURE 1] showed in all samples a heterogenous surface full of crystal line structures with more width at the dentin – Bio dentin interphase.^[1]

Endocem MTA [FIGURE 2] showed better homogeneity than Bio dentine in the bio material core and lesser gap at the dentin – Endocem interphase in comparison to the dentin – Bio dentin interphase.

Nano MTA [FIGURE 3] showed the best homogeneity within the biomaterial core, and very less abrupt changes in the dentin-Nano MTA interphase among all the three groups. In Nano MTA [FIGURE 3], a constant and uniform nonporous Gray image was observed, which might be ascribed to a more proper hydration of products and good interlocking of the crystal compounds.

An increase in the surface area of the material obtained by nano-sizing the powder particles would enhance the hydration phase of cement particles and better interlocking of powder particles to create a cement structure with better integrity. It is seen that in mixing and working phase, as more particles are involved in reaction with the hydration phase, a less porous set material is formed. Also, Nano MTA's particle size has a great impact on the extent of particles penetrating the dentinal tubules, thereby decreasing the gap between Nano MTA and root dentin wall.^[3]

The increased width between the Bio dentin and Endocem MTA [FIGURE 2] to the root canal wall could be due to the larger particle size of Bio dentin and Endocem MTA particles compared to Nano MTA that decreases its penetration to the root canal dentin thereby, decreasing the marginal adaptation.

The statistical analysis using ANOVA and Mann Whitney post hoc tests were done.

The test results demonstrated that the mean Marginal Adaptation gap values for Bio dentin group was 26.490 ± 12.728 , for Endocem MTA group was 6.125 ± 2.080 and for Nano MTA group was 4.198 ± 0.929 . This mean difference in the Marginal Adaptation gap values between 3 groups was statistically significant at $P < 0.001$.

The mean differences in marginal adaptation gap values between the three groups showed that Nano MTA group showed significantly least marginal adaptation gap values as compared to the Endocem MTA and the Bio dentin group at $P < 0.001$ & $P = 0.03$ respectively. This was then followed by, Endocem MTA group showing significantly lesser mean marginal adaptation gap as compared to the Bio dentin group at $P < 0.001$. The larger marginal adaptation gap was shown by the Bio dentine group among all the three groups at $P < 0.001$ & $P = 0.03$ respectively.

This infers that Nano MTA showed significantly better marginal adaptation to the root canal dentin as compared to other groups, which was followed by the Endocem MTA group and the least marginal adaptation was shown by the Bio dentin group. This result is in agreement with various studies, which reported that nano MTA presented excellent sealing ability, and demonstrated its superiority in comparison with other commonly used root-end filling materials, when viewed under scanning electron microscope.^[28]

However, it is worth mentioning that further clinical studies are necessary for the properties of Bio dentine, Endocem MTA and Nano MTA to assess its use as an ideal root-end filling material apart from its marginal adaptability.

Conclusion

Within the limitations of this study, it was concluded that all the three endodontic biomaterials showed good

marginal adaptation with the root canal dentin when used as apical plugs on examination under scanning electron microscope.

However, the most marginal adaptation of the apical plugs with the root canal wall was seen in the case of Nano MTA, followed by Endocem MTA which were statistically significant. Least marginal adaptation of the apical plugs with the root canal wall was seen in the Bio dentine groups.

However, further research is required for properties of Nano MTA and Endocem MTA to assess its use as an ideal root-end filling material apart from its marginal adaptability.

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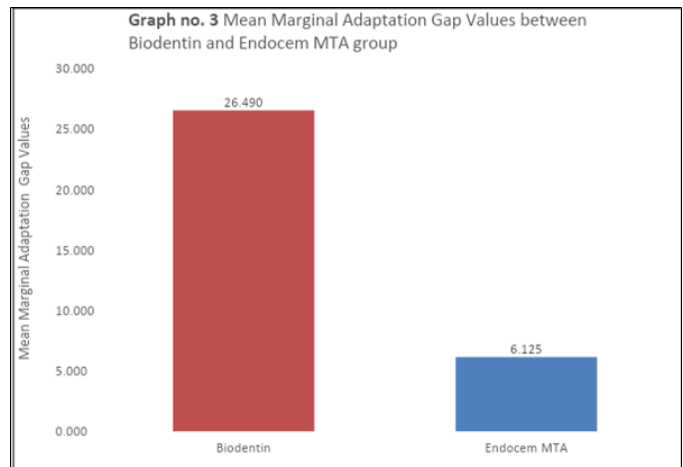
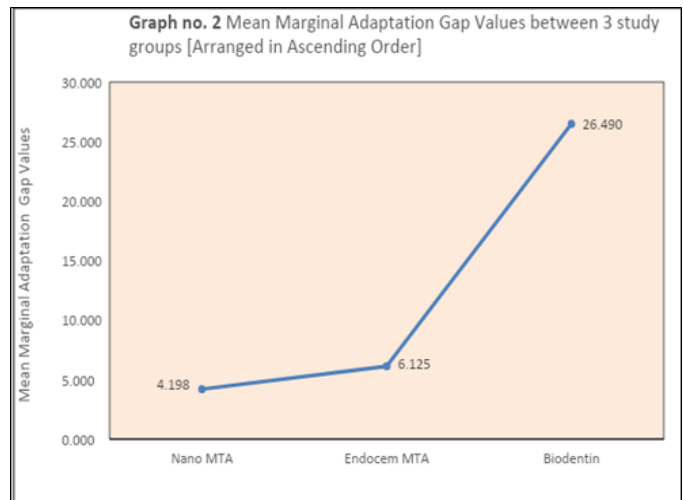
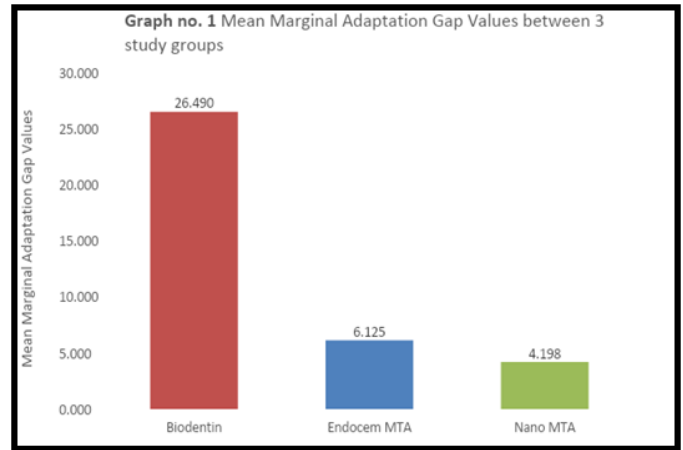
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Legend Table and Figure

Table1: Comparison of mean Marginal Adaptation Gap Values between 3 study groups using One-way ANOVA Test						
Groups	N	Mean	SD	Min	Max	P-Value
Bio dentin	10	26.49	12.72	9.0	44.8	<0.001*
Endocem MTA	10	6.125	2.080	3.2	9.60	
Nano MTA	10	4.198	0.929	3.2	6.13	

N: Number of samples, SD: Standard deviation.

Table no. 2 Multiple comparison of mean difference in Marginal Adaptation Gap Values between groups using Mann Whitney Post hoc Test					
(I) Groups	(J) Groups	Mean Diff. (I-J)	95% CI for the Diff.		P-Value
			Lower	Upper	
Bio dentin	Endocem MTA	20.365	12.087	28.643	<0.001*
	Nano MTA	22.292	14.014	30.570	<0.001*
Endocem MTA	Nano MTA	1.927	-6.351	10.205	0.03*



List of abbreviations

CDC	Center for Disease Control and Prevention
MTA	Mineral Trioxide Aggregate
EDTA	Ethylenediamine tetraacetic Acid
NaOCl	Sodium Hypochlorite
PBS	Phosphate buffer solution
IRM	Intermediate Restorative Material
mm	Millimetre
CEM	Calcium Enriched Mixture
nWMTA	Nano White Mineral Trioxide Aggregate
Fig	Figure
mL	milliliter
n	Number
°C	Degree Celsius
%	Percentage
CSCs	Calcium Silicate Cements
PDL	Periodontal ligament