An Invitro Comparative Evaluation of the Sealing Ability of Five Different Root End Filling Materials Using High Copper Amalgam, Resin Modified Glass Ionomer Cement, Cermet Cement, Mineral Trioxide Aggregate and Biodentine: An Analysis by Confocal Laser Microscopy

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Abstract

Aim: To evaluate the sealing ability of different root end filling material by assessing the apical microleakage from the prepared root ends of the teeth in order to find out which material provides a better sealing ability. Root canal system is very complex in nature due to presence of lateral canal and ramifications at the apical and of the route that's why it is very difficult to clean it completely. Therefore, root canal cannot always be treated using an Ortho grade approach. Upon failure of primary and romantic therapy one can choose either to Retreat the tooth non surgical with an Ortho grade route feeling or surgically with apicoectomy and a retrograde root and filling. The procedure can be performed by simply cutting of the apical portion of the route then playing the cut surface and preparation of apical area followed by selling it with suitable root end filling material in order to establish fluid tight seal to prevent the passage of microorganisms or there by products into periapical tissues.

Method: Methods 90 human maxillary incisors were collected and decorated at the level of cement enamel junction using high speed cutting disc. After BMP this sample were obturated with lateral condensation technique. later on apical 3 mm resection of roots were done using cutting disc followed by retrograde cavity preparation of 3 mm deep with ultrasonic tips. The sample were divided into 6 groups depending upon different route end filling material (amalgam, RMGIC, cermet, MTA, biodentine) and one control group the sample were then coated with 2 layers of nail varnish followed by layer of approximate 2 mm of sticky wax to the external surface of each root except for the apical section. In case of control group apical section of the sample were covered with sticky wax in order to prevent the dye preparation later all the roots are stored in 100% humidity for 24 hours. The roots were later on totally immersed in solution of rhodium b fluorescent dye for 24 hours. Then using a diamond disc each root was longitudinally section and observed under confocal laser scanning microscope to check the extent of Dye penetration. The results were subjected to statistical analysis.

Results: The main dye penetration of different groups for group 1 control group 0.00 ± 0.00 mm group 2 Silvar amalgam 3.00±0.00 mm group 3 RMGIC 1.84±0.26mm group 4 cerment cement 1.83± (.25mm) Group 5 MTA 1.25±.12 mm group 6 Biodentine .26±.21 mm thus among the various root end filling material tested the new material biodentine exhibited least dye preparation with better marginal sealing ability then other material tested.

Interpretation and observation on the basis of result obtained it can be concluded that the newer material biodentine exhibit least apical microleakage and silver amalgam should maximum biodentine showed promising result with least dye penetration because setting time is lower that is 12 minutes and Tag like structures are formed composed of calcium phosphate deposit between the tooth and root end filling material.

Keywords: Microleakage, Root end Feeling Materials, Rhodium B, Biodentine, MTA

INTRODUCTION

Periradicular lesions develop when root canals are exposed to the oral flora. The root canal system can harbor several species of bacteria as well as their toxins and by products. Ingress of these irritants from the root canal system into periradicular tissues result in the formation of periradicular lesions. Root canal system is very complex in nature due to presence of lateral canals and ramifications at the apical end of the root that's why it is very difficult to clean it completely. Therefore, root canals cannot always be treated using an orthograde approach.[1]

Upon failure of primary endodontic therapy, one can choose either to re-treat the tooth non-surgically with an orthograde root filling or surgically with apicoectomy and a retrograde root end filling. The rationale behind the removal of most apical 3mm of the root is to remove 98% of apical ramifications and 93% of lateral canals in order to achieve apical bone healing.[2]

Various studies have shown that the best outcome of retreatment is by surgical approach which is achieved by apical cavity preparation and placing the root end filling material.[3] The properties of an ideal root-end filling material is as follows: The material should adhere to tooth tissue and "seal" the root-end 3-dimensionally. It should inhibit the growth of pathogenic microorganisms, stable under moisture, well tolerated by periradicular tissues with no inflammatory reactions; nontoxic, should stimulate the regeneration of normal periodontium. It should be easily distinguishable on radiographs and have a long shelf life.[4]

Micro leakage is a common phenomenon in which the passage of bacteria, fluids and chemical substances between the root structure and filling material takes place, hindering the healing of the lesions.[5] Therefore, there are many methods that can be employed to evaluate the micro leakage of materials like passive penetration of dyes, fluid perfusion tests, bacterial penetration models and capillary flow porometry, with the help of which sealing ability of materials to resist micro leakage through the entire thickness of the material can be checked in order to find out an adequate root end filling material with best possible apical seal to prevent the surgical endodontic failure.

Many dental materials like Amalgam, IRM, Composite resins, MTA etc. have been tested and are widely used as root-end endodontic filling materials with high success rates in clinical trials.[6] Recently, Biodentine has been proposed as a root end filling material which is mainly composed of tricalcium silicate, calcium carbonate and zirconium oxide as the radiopacifier, whilst its liquid contains calcium chloride as the setting accelerator and water reducing agent. It has reduced setting time, better handling and mechanical properties with better marginal seal.[7]

Therefore, this study is designed to evaluate the sealing ability of different root end filling materials by assessing their apical microleakage from the prepared root ends of the teeth in order to find out which material provides a better sealing ability by confocal laser microscopy.

MATERIALS AND METHODS

Methodology:

90 freshly extracted sound human maxillary incisors with mature apices were collected and stored in 3% hydrogen peroxide solution until use.

Sample Preparation

The teeth were decoronated at the level of cemento-enamel junction with a low speed diamond disc using a straight handpiece (marathon) and cervical preflaring was done with Gates Glidden drills #2 & #3. The working length was recorded with help of K files (Mani inc.) which were introduced in the root canals until the tip was visible from the apex and then pulled back 1 mm. Biomechanical preparation was done with hand K files (Mani inc.) upto size #40K file by standardized technique. Copious irrigation with 3% sodium hypochlorite was done all throughout the procedure. Then, the canals were dried using absorbent points and obturation was done with 2% gutta percha points and zinc oxide eugenol sealer using the lateral compaction technique. Radiographs were taken to confirm the complete filling of the root canal. After that, the coronal access cavities were sealed with Type II Glass Ionomer Cement (Shofu inc, Japan). Then, all the samples were kept in incubator at 37^{o}c for 24 hours.

Later on, all the samples were divided into one control group and five experimental groups containing 15 teeth each. The apical 3 mm of root ends were resected at approximately 90^{0} to the long axis of the tooth with diamond disc using straight handpiece. Then, 3 mm retrograde cavity was prepared with ultrasonic tip (ED11) in all the samples.

Group I (Control group): In this group**,** all 15 samples were coated with two coats of nail varnish and sticky wax to prevent the penetration of dye solution.

Group II (High copper Amalgam): In this group retrograde filling was done with High Copper Amalgam.

Group III (RMGIC): In this group, RMGIC in a ratio of 3:1 powder liquid was taken according to the manufacturer's instructions and mixed on a paper pad with plastic spatula by folding method for 10-15 sec to create a glossy consistency. After placement of material in cavities curing was done for 20 seconds by light curing unit (WoodPecker LED unit) with an intensity of $1200mV/cm^2$.

Group IV (Cermet Cement): Silver reinforced GIC was mixed in a ratio of 7:1 powder: water according to the manufacturer's instructions on a mixing pad with plastic spatula for 40-50 seconds and was filled in all the cavities.

Group V (Mineral Trioxide Aggregate): MTA was prepared by mixing powder and liquid in a ratio of 3:1 on the mixing slab until a creamy consistency was achieved which was then retrofilled in the prepared cavities and condensed.

Group VI (Biodentine): Biodentine was prepared by first adding 5 drops of its liquid into the capsule containing powder and then mixed in mixing unit (R-4C, Remi Lab instruments, Mumbai, India) for 30 seconds, at 4300 oscillations per minute. Later on, it was used to retrofill all the prepared cavities. Then, all the samples were wrapped in wet gauze pieces and stored in 100% humidity for 24 hours in an incubator at 37 ^{0}C.

Preparation of Samples for Dye Immersion

After 24 hours, two coats of nail varnish were applied to the external surfaces of each sample of experimental groups. The prepared root surfaces were carefully coated with finger nail varnish so that only the retro filling material remained exposed. These samples were then covered externally with a layer of approximately 2mm of sticky wax except for the apical section. Then, all the samples of control and experimental group were totally immersed in a solution of Rhodamine B fluorescent dye for 24 hours.

Observation under Confocal Laser Microscope

After Dye immersion, the samples were washed under running water, dried and the external layers of sticky wax and nail varnish were removed from each sample. Later on, using a diamond disc, each sample was longitudinally sectioned in order to prepare them for examination under Confocal Laser Scanning Microscope to check the extent of dye penetration in all the samples using oil immersion objectives in conjunction with a green filter (wavelength 546 nm).

STATISTICAL ANALYSIS

Descriptive data are presented as mean ± SD and Range values. One-way ANOVA was used for multiple group comparisons followed by Post Hoc Tukey's HSD Test for differences among mean.

RESULTS

The present in-vitro study was designed to evaluate the apical microleakage of different root end filling materials (Amalgam, RMGIC, Cermet cement, MTA, Biodentine) by dye penetration method under Confocal Laser Scanning Microscope.

Among the various root end filling materials tested, the newer material Biodentine exhibited least dye penetration with better marginal sealing ability than other materials.

This showed that the **Group VI** (Biodentine) had the least dye penetration of **0.26±(0.21) mm** as compared to other materials. Whereas **Group V** (MTA) showed the mean dye penetration of **1.25±(0.12) mm** followed by **Group IV** (Cermet Cement) **1.83±(0.25) mm** and **Group III** (RMGIC) **1.84±(0.26) mm** with **Group II** (Amalgam) showing the highest amount of mean dye penetration of **3.00±(0.00) mm.** In **Group I** (Negative control) dye could not penetrate and showed the mean value of **0.00±(0.00) mm.**

Group VI < Group V < Group IV ≤ Group III < Group II

DISCUSSION

Radicular lesions develop only when root canals are exposed to the oral flora, and the root canal system has the capacity to harbor several species of bacteria as well as their toxins and by products. Engress of these irritants from the root canal system into radicular tissues results in the formation of radicular lesions. Due to the complexity of the root canal system, it is not possible to completely clean it.[1]

When non-surgical attempts prove unsuccessful or are contraindicated then, surgical endodontic therapy is needed to save the tooth. So, the goal of periradicular surgery is to gain access to the affected area, exposed the involved apex, remove the diseased tissue, resect the apical end of the root, prepare a class I cavity and insert a root end filling material that can stimulate regeneration of the periodontium. The formation of new cementum on the surgically exposed root surface and on the root end filling material is essential to the regeneration of the periodontium.[8]

A number of materials have been evaluated for retrograde root-end fillings. They include amalgam, gutta-percha, zinc oxide eugenol cements, composite resins, glass ionomer, polycarboxylate cements, ethoxybenzoic acid (EBA) cement, and mineral trioxide aggregate (MTA). But no material has yet been found to meet all ideal requirements.[9] In recent years, Biodentine have been introduced with the aim of overcoming some of the disadvantages of other retrograde filling materials.[7]

In the present study, all the root end filling materials showed some amount of dye penetration except the control group in which no dye penetration was allowed by sealing the resected apical section of the roots. Hence, from this observation we can conclude that the recent material Biodentine **(Group VI)** exhibited least apical microleakage **(0.26±0.21 mm)** among all the other materials tested (Amalgam, RMGIC, Cermet Cement, MTA).

Pathak S compared and evaluated the best sealing ability of four different root end filling materials i.e. GIC, IRM, MTA Angelus and Biodentine using Scanning Electron Microscope and Stereomicroscope. The results showed that amongst all the groups, Biodentine showed with least width of gap values, whereas IRM showed maximum values of gap amongst the tested samples. The probable reasons for better marginal adaptation and less microleakage seen in Biodentine may be attributed to the formation of tag like structures composed of Calcium or Phosphate rich crystalline deposits which increases over time hence minimizing the gap between tooth and retro filled material.[10]

Nanjappa AS et al compared and evaluated the sealing ability of different root-end filling materials Mineral Trioxide Aggregate (MTA), Biodentine and Chitra-Calcium Phosphate Cement (CPC) observed under Confocal Laser Scanning Microscope using Rhodamine B dye. They found that Biodentine showed least microleakage followed by MTA and Chitra-CPC. According to them, biodentine showed better result because the setting time of biodentine is 12 min as compared to MTA whose setting time is 2 hours 45 mins and it also bonds chemomechanically with the tooth with high compressive and flexural strength.[11]

Hindlekar A et al evaluated the sealing ability of three-root end filling materials i.e Mineral Trioxide Aggregate, Biodentine and Injectable Glass Ionomer Cement in blood and saliva contaminated conditions by using 50% Silver Nitrate dye. The results showed that there was no significant difference between the sealing ability of MTA and Biodentine in contaminated cavities. However, both MTA and Biodentine showed better sealing ability in comparison with Injectable Glass Ionomer cement.[12]

Kokate SR et al comparatively evaluate stereomicroscopically the microleakage of three root end filling materials Mineral Trioxide Aggregate (MTA), Glass Inomer Cement (GIC) & Biodentine using dye penetration. They found that the microleakage was significantly less in Biodentine as compared to others. Biodentine is a calcium silicate based cement, it has increased physico-chemical properties like short setting time, high mechanical strength which make it clinically easy to handle and compatible.[13]

Thus, the result of the present study were in accordance with the above studies which concluded that Group VI (Biodentine) had significantly lowest mean dye penetration. This may be due to the lower setting time i.e 12 mins and formation of tag like structures composed of Calcium or Phosphate rich crystalline deposits between the tooth and root end filling materials.

After Biodentine, **Group V** Mineral Trioxide Aggregate (MTA) showed lesser microleakage (**1.25±0.12mm**) as compared to RMGIC, Cermet Cement and Amalgam. MTA is a calcium silicate based bioactive material which when hydrated results in a colloidal gel that solidifies in the mineralized structure of the tooth (Torabinejad, et al.,1993), leading to a marginally satisfactory adaptation due to the possible expansion of this material in a humid environment.[14]

Gundam S et al compared the marginal adaption of Mineral Trioxide Aggregate (MTA), Glass Ionomer Cement (GIC) and Intermediate Restorative Material (IRM) as root-end filling materials in extracted human teeth using Scanning Electron Microscope (SEM). They found MTA with better marginal adaptation as compared to IRM and GIC. The increased sealing ability of MTA is attributable to its hydrophilic nature and expansion when set in a moist environment.[15]

Mohammad F et al compared the sealing ability of Resilon, MTA and Gutta-Percha as root end filling materials by dye penetration which was later on measured with a Stereomicroscope. They found that the resected gutta-percha showed significantly more leakage than MTA. The leakage in Resilon group was more than MTA group and slightly lesser than Gutta-Percha. Thus, MTA has better sealing ability it produces a better mechanical seal in moist environment with better marginal adaptation.[16]

Thus, the results of the present study were in accordance with the above studies which concluded that Group V (MTA) had significantly lower mean dye penetration. This may be due to its hydrophilic nature and expand when allow to set under moisture which tends to fill the gap between the dentin and root end filling material.

Therefore, after analyzing the results of the present study, the newer material Biodentine had shown promising results as a root end filling material. It showed least microleakage and better marginal adaptation as compared to other materials tested (Mineral Trioxide Aggregate, RMGIC, Cermet Cement, Amalgam).

CONCLUSION

Under the limitations of the current in vitro study, following conclusion can be drawn:

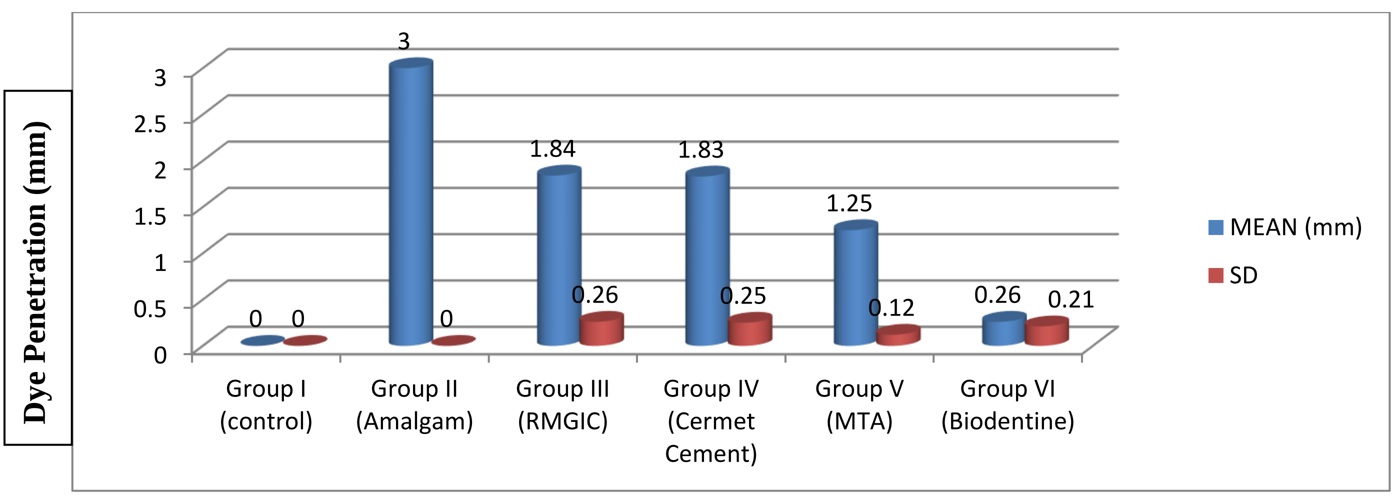
- ✓ **Group VI (Biodentine)** which is the newer root end filling material exhibited the lowest mean dye penetration as compared to the other root end filling materials tested (p<0.001).
- ✓ **Group II (Amalgam)** showed highest Dye penetration with worst marginal sealing ability, so such material should not be preferred for retrograde filling in periapical surgeries.
- ✓ **Group V (MTA)** showed dye penetration next to **Group VI,** so it can be stated that the MTA can be used as second preference next to Biodentine.
- ✓ **Group III (RMGIC)** and **Group IV (Cermet Cement)** showed insignificant difference in the dye penetration as they both are the modifications of conventional GIC but still failed to show better sealing ability as compared to MTA and Biodentine.

But in the clinical conditions with the presence of dynamic nature of periapical tissue which could be different from the in vitro situation. Hence, it is recommended that further research be employed in this area to collect evidence based data to support this in vitro study.

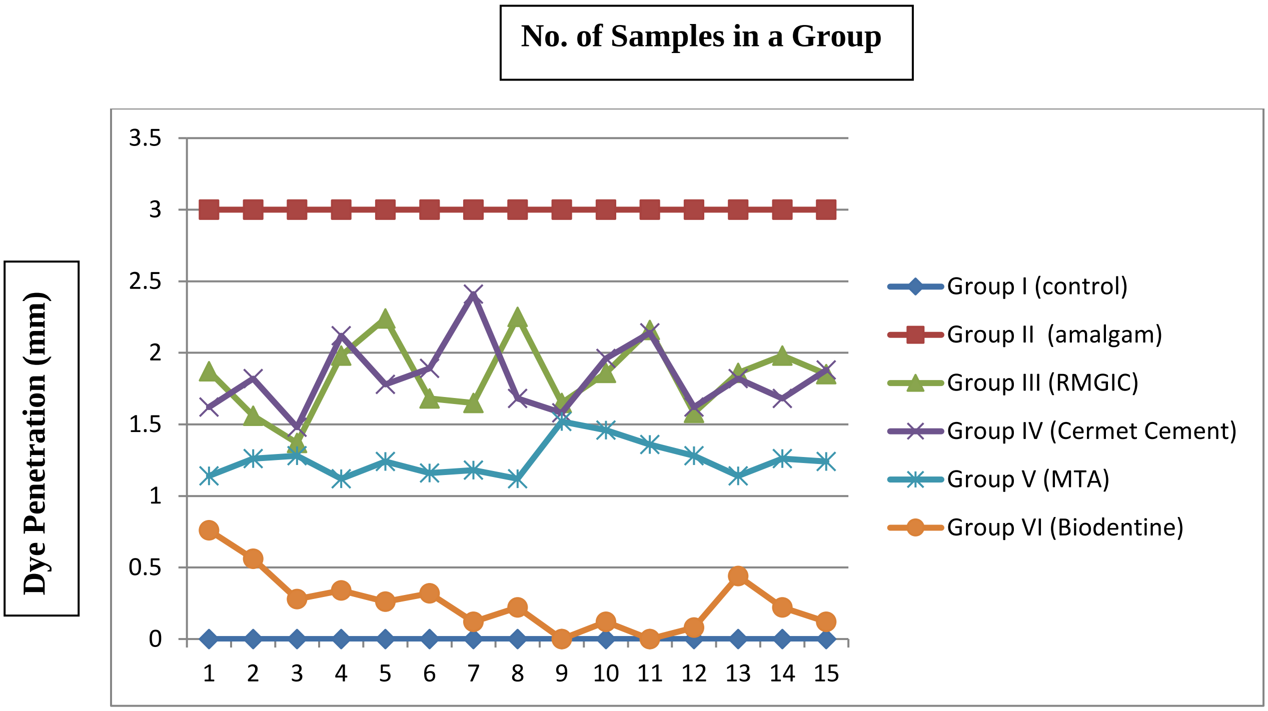
So, our present study recommends that newer material Biodentine had shown better promising results with the better marginal adaptability and least apical microleakage which can be used as a root end filling material.

Table 1: Demonstrates the intra group comparison of mean and standard deviation values of dye penetration at the apical end of different root end filling materials tested using One Way ANOVA.

GROUPS	N	MEAN (mm)	SD	MIN	MAX	P VALUE
Group I (control)	**15**	**0.00**	**0.00**	**0.00**	**0.00**	**<.0001**
Group II (amalgam)	**15**	**3.00**	**0.00**	**3.00**	**3.00**	
Group III (RMGIC)	**15**	**1.84**	**0.26**	**1.37**	**2.25**	
Group IV (Cermet Cement)	**15**	**1.83**	**0.25**	**1.48**	**2.41**	
Group V (MTA)	**15**	**1.25**	**0.12**	**1.12**	**1.52**	
Group VI (Biodentine)	**15**	**0.26**	**0.21**	**0.00**	**0.76**	



Graph 1 (Bar Diagram): Depicts the intra group comparison of mean and standard deviation values of dye penetration at the apical end of different root end filling materials tested.



Graph 2 (Line Diagram): Depicts the individual values of dye penetration of all the samples of different root end filling materials tested.

Table 2: Demonstrates the inter group comparison of mean dye penetration at the apical end of different root end filling materials tested using Post Hoc Tukey's Test.

GROUPS	Q STATISTICS	INFERENCE
CONTROL vs AMALGAM	**65.9045**	**** p<0.01**
CONTROL vs RMGIC	**40.3336**	**** p<0.01**
CONTROL vs CERMET CEMENT	**40.2457**	**** p<0.01**
CONTROL vs MTA	**27.4749**	**** p<0.01**
CONTROL vs BIODENTINE	**5.6239**	**** p<0.01**
AMALGAM vs RMGIC	**25.571**	**** p<0.01**
AMALGAM vs CERMET CEMENT	**25.6588**	**** p<0.01**
AMALGAM vs MTA	**38.4297**	**** p<0.01**
AMALGAM vs BIODENTINE	**60.2807**	**** p<0.01**
RMGIC vs CERMET CEMENT	**0.0879**	**insignificant**
RMGIC vs MTA	**12.8587**	**** p<0.01**
RMGIC vs BIODENTINE	**34.7097**	**** p<0.01**
CERMET CEMENT vs MTA	**12.7708**	**** p<0.01**
CERMET CEMENT vs BIODENTINE	**34.6218**	**** p<0.01**
MTA vs BIODENTINE	**21.851**	**** p<0.01**

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