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Comparison of Sealing Ability of Three Different Retrograde Filling Materials- A Confocal Laser Scanning Microscopic Study

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Abstract: The purpose of this investigation was to evaluate the sealing ability of three different retrograde filling materials like Intermediate Restorative Material, Mineral Trioxide Aggregate and Bioaggregate by dye penetration method using confocal laser scanning microscopy. Here, 32 human maxillary central incisors were selected and then decoronated for the instrumention followed by the obturation of roots finally with gutta-percha and resin sealer. Later they were divided randomly into 2 control groups of one sample each and 3 experimental groups of 10 sample each. In the negative control group, sample was kept without any further preparation whereas in the positive control and experimental groups, roots were resected 3 mm from the apex. Then the root-end cavities were prepared using an ultrasonic tip and no retrograde filling was done in positive control, whereas 3 experimental group roots were filled with one of the following materials i.e Intermediate restorative material (IRM), Mineral trioxide aggregate (MTA) and Bioaggregate (BA). There after apical leakage was assessed using Dye penetration technique in conjunction with confocal laser scanning microscope (CLSM) followed by One-way analysis of variance (ANOVA) and Tukey's test. Mean dye leakage of IRM was significantly higher when compared with WMTA and BA materials (P<0.001). Among all three materials the dye leakage was less in WMTA.
Keywords: White Mineral Trioxide Aggregate, Bioaggregate, Intermediate Restorative Material, Rhodamine dye penetration method, confocal laser scanning microscope.

INTRODUCTION

The success of any root canal therapy dependents on its thorough debridement, sterilization and complete obturation where the violation of one of any these principal elements results in periapical invasion of tissues by bacteria and their byproducts causing the failure of the treatment thereby recommending retrograde filling, obturation, surgical sealing of the root canal after the exposure of the involved apex, apical root resection, retropreparation[1,2].

The main objective of the root end filling material is to provide an apical seal that prevents the movement of bacteria and diffusion of their products from the root canal system into the periapical tissues.[3] Throughout the dental history, a wide variety of materials have been used for retrograde fillings, however, till to date, no root-end filling material satisfies the ideal requirements, therefore, the development of a novel root end filling material is a constant concern for many researchers.

Thus, our present invitro study aims at evaluating adaptation and sealing ability of three different retrograde materials namely Intermediate restorative material, Mineral trioxide aggregate and relatively new material Bioaggregate using[4] Confocal laser scanning microscope and fluorescent Rhodamine B dye by visualizing dye penetration.

MATERIALS AND METHODS

Here, 32 fresh human maxillary central incisors extracted for periodontal and orthodontic reasons, were selected so as to standardize the apical root configuration of all the samples and reduce the variations in the root canal anatomy.Then, the decoronation of the selected teeth was done at cemento-enamel junction using a diamond disc and a straight hand piece under copious water irrigation. Later an ISO 15 K file was introduced into the canal to check the patency and the working length was determined followed by cleaning and shaping using standard step-back technique until reaching a master apical size 40 where the canals were irrigated with 10 ml of 3.0% Sodium hypochlorite in between successive files and the smear layer was removed by irrigation with 10 ml of 17% EDTA solution with a final rinse with 10 ml of normal saline. All canals were dried with paper points and obturated by lateral condensation technique using AH plus sealer and guttapercha followed by the radiographic confirmation of the quality of obturation where the canal orifices were sealed with Glass Ionomer cement (Type II), the roots were then wrapped in moist gauze and kept at room temperature and 100% relative humidity for one week.

Now, the apical root resections were performed at 90º angle to the long axis of the root using cross cut fissure bur No.556 removing 3 mm of the apex. The rationale for resecting 3mm of root apex in this study was to remove all the accessory canals present apically and prevent reinfection as 98% of apical canal anomalies and 93% of lateral canals system ramifications occur in the apical 3 mm[5, 6].

Then the retrograde cavity was prepared using S12/90 D retrotip powered by satelec ultrasonic unit with a technique of intermittent and minimal pressure with an in and out motion at the lowest power setting and internal water cooling as recommended by the manufacturer where the depth of the cavity prepared was 3mm followed by the irrigation of the cavities with saline and dried before filling with the test material.

Grouping of samples

The samples were then randomly divided into three experimental groups of 10 roots each i.e. group I, II, III according to the material used and into a positive control group of IV without retro filling and a negative control group of V without further retropreparation and retro filling whereas the experimental groups are filled with WMTA, Bioaggregate and IRM respectively.

Procedure for placement of retrofilling material

The materials were mixed according to the manufacturer's instructions and the retrograde cavities were filled using a Messing's carrier. The excess was removed with the probe and the fillings were wiped with a cotton pellet. All the specimens were wrapped in wet gauze and placed in an incubator at 37º C for 48 hours to allow complete set of the root-end filling materials. Then the specimens were coated with nail varnish except at the apical 3mm in test groups and positive control group whereas in negative control group entire tooth surface including the canal orifice and the apical foramen was coated with nail varnish to prevent the dye leakage into the root canal system, later all the samples were allowed to dry.

Now to evaluate apical leakage, the apical third of each root was suspended in 0.1% Rhodamine B dye for 24 hours. Then using a slow speed diamond disc under water coolant each root was longitudinally sectioned into two halves and was oriented and secured to a glass slide. Each specimen was then examined to evaluate the extent of linear dye penetration using Confocal Laser Scanning microscope 10x magnification where the

Rhodamine B dye gave a red-orange fluorescence when excited with yellow-green light of 561nm wavelength. The amount of dye penetration was measured in µm using the ZEISS LSM IMAGE BROWSER SOFTWARE (Version 4.2.0.121) and statistical analysis was done using Social Sciences (SPSS) program version 20 by one-way analysis of variance (ANOVA) and Tukey's test where the results with P value < 0.05 were considered statistically significant.

OBSERVATIONS AND RESULTS

All the samples in the experimental groups showed some amount of dye leakage. The sample of the positive control group showed maximum dye leakage throughout the retrograde cavities (Fig 1), thus confirming that root end filling material was necessary to prevent micro leakage; in contrast, sample in the negative control group showed no dye leakage (Fig 2), and thus confirming that nail polish prevented dye leakage.

Interpretation

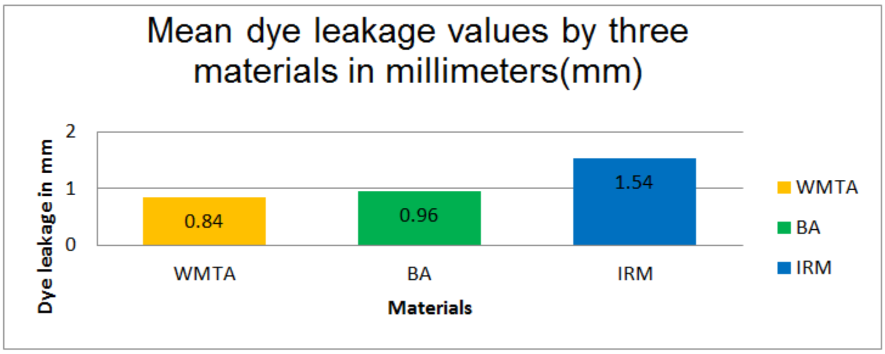
Tukey's multiple Post Hoc test statistics revealed that mean dye leakage of IRM was significantly higher when compared with WMTA and BA materials (P<0.001) (Table 1,2; Fig 3,4). There was no statistically significant difference between BA and WMTA (P>0.001) (Table 1, 2; Fig 3) and among all the three materials the dye leakage was less in WMTA (Graph 1).

Table-I: Comparison of Dye leakage (mm) for three different materials

Material	Summary Of Dye Leakage			F Test Ratio	P-Value
	Mean	S.D	N		
WMTA	0.84	0.28	10	11.02	0.0003
BA	0.96	0.50	10		
IRM	1.54	0.22	10		
Total	1.11	0.46	30		

Table-2: Intergroup comparison of three groups with respect to linear dye leakage (in mm) by Tukey's multiple Post hoc comparisons

Dye leakage	Mean Diff	S.E	Tukey t	Tukey P>|t|	[95% Conf. Interval]	
BA vs WMTA	0.12	0.16	0.75	0.74	-0.28	0.52
IRM vs WMTA	0.70	0.16	4.39	<0.001	0.30	1.10
IRM vs BA	0.58	0.16	3.64	<0.001	0.18	0.98



Graph-1: Evaluation of mean dye leakage values of three retrograde filling materials

Note: X-axis represents mean dye leakage values; Y-axis represents the three materials used

NOTE: Orange red fluorescence indicates dye penetration

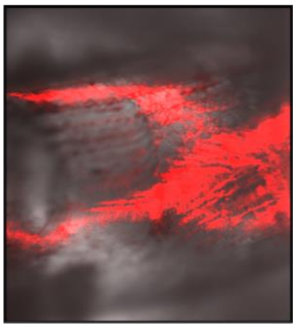


Fig-1: Confocal laser scanning microscopic image of positive control group sample

Fig-2: Confocal laser scanning microscopic image of negative control group sample

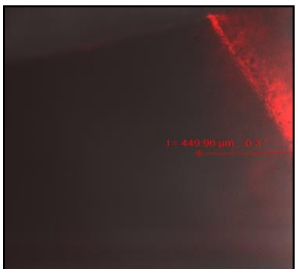


Fig-3: Confocal laser scanning microscopic images of dye penetration with WMTA

Fig- 4: Confocal laser scanning microscopic images of dye penetration with BIOAGGREGATE

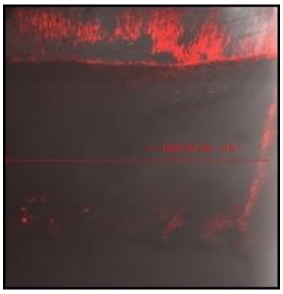


Fig-5: Confocal laser scanning microscopic images of dye penetration with IRM

DISCUSSION

The root canal system has the capacity to harbor several species of bacteria as well as their toxins and by-products whenever they are exposed to oral flora causing many of the endodontic radicular lesions, secondary to egression of these irritants into radicular tissues, resulting in the formation of periapical pathosis. Thereby, the removal of these irritants and total obliteration of the root canal system in three dimensions are the main goals of nonsurgical root canal therapy.

However, because of the complexity of these root canal system and inability to clean it using present techniques and instruments, they cannot be treated always adequately using a nonsurgical orthograde approach then retreatment of unsuccessful cases is the preferred treatment of choice and usually results in a successful outcome. When nonsurgical attempts prove unsuccessful and/or contraindicated, surgical endodontic therapy is needed to save the tooth.

The endodontic surgery usually comprises of exposure of the involved apex, resection of the apical end of the root, preparation of retrocavity and insertion of a root end filling material[4] to achieve an adequate apical seal which inhibits the leakage of residual irritants from the root canal into the periradicular tissues[7, 8]. If the apical seal is inadequate, the residual microorganisms can cause re-infection, thereby inhibiting healing of the periapical tissues[4,7,8].

Till today, numerous substances have been used as root-end filling materials and the choice of these materials could be determined by their apical sealability, biocompatibility, handling properties and long term clinical success records[4] which include materials like newer modifications of Zinc Oxide Eugenol, compounds such as Intermediate restorative material, Mineral Trioxide Aggregate. Recently, Bioaggregate, a new bioceramic based material was introduced into the market, which is recommended for perforation repair and root end filling.

Thus the present study aims at evaluating the sealing ability of three different retrograde filling materials like Mineral Trioxide Aggregate, Bioaggregate and Intermediate Restorative Material by dye penetration method after the successful completion of orthograde treatment followed by apisection and retrograde filling, where they are grouped equally into Group I, II, III each composed of 10 roots followed by comparison with a positive control group IV and a negative control group V each composed of 1 root.

A plethora of root end filling materials exists from many years where Gartner and Dorn, has suggested certain requirements for an ideal root end filling material [9,10]. However, till to date no material has been found which has all or most of the ideal properties of a root end filling material. Hence, the quest for the best retro filling material still continues where historically, zinc oxide-eugenol cements have been used extensively as root end filling materials from the times of its introduction since 1890's out of which IRM and Super EBA are most commonly used and IRM was first described as a root-end filling material in 1990 by Dorn & Gartner.

In the present study, Intermediate restorative material was selected because it is one of the oldest materials used with reasonable success and found that it leaked significantly more than that of WMTA and BA. Bioaggregate it is a new generation of a root canal repair filling material where the manufacturer claims that it is produced under controlled conditions to form contamination free ceramic nano-particles by nano-technology which upon reaction with water produces biocompatible and aluminum-free ceramic biomaterials. The composition of BA is similar to that of MTA, but the major difference between MTA and BA is that BA contains tantalum oxide as a radiopacifier and has no aluminum content [11] and present study showed less dye leakage with Bioaggregate than IRM which was statistically significant but slightly more dye leakage than WMTA with no statistically significant difference.

In a study conducted by Leal *et al*. Ceramicrete, Bioaggregate and MTA were compared to prevent glucose leakage through root-end fillings[12].They concluded that Bioaggregate displayed similar leakage results to white MTA when used as root-end filling material, which is in accordance with our study but both the studies are contradictory to El Sayed and Saeed who evaluated and compared in vitro sealing ability of Bioaggregate versus amalgam, IRM and MTA which reported that Bioaggregate showed least amount of microleakage when compared to MTA [3] and this difference may be attributed to difference in method of assessment of dye leakage. Thus, Bioaggregate was selected in this invitro study, as it is a relatively new material, and there are only few studies available about this material.

Mineral trioxide aggregate (MTA), it was developed by Dr. M. Torabinejad and Dean J. White in the early 1990s and was used as a potential root-end filling material or as a repair material for lateral root perforations where White MTA was developed and introduced in 2002 as an aesthetic alternative to Gray MTA [13] as later one has potential to cause discoloration of overlying tissues. The sealing ability of MTA has shown to be superior, unaffected by blood contamination and moisture during its packing in various studies with no significant difference in its retention when a dry or wet cotton pellet was used [14]. In a study conducted to compare amalgam, Super EBA and MTA, it was concluded that MTA provides better adaptation and sealing [15], which is in accordance with our study as well as another clinical study comparing MTA and IRM, as it showed a higher success rate due to its good sealing properties, bioactivity, and its potential to stimulate cementogenesis [16] resulting from its hydrophilic properties and formation of interfacial layer between material and dentin. It was found that further hydration of MTA powder by moisture can result in an increase in the compressive strength and decrease leakage.

In our study, the quality of the apical seal obtained by root end filling materials has been assessed by the degree of dye leakage as it is the easiest method to screen new restorative filling materials and when a filling material does not allow penetration of small molecules, it has the potential to prevent leakage of larger substances such as bacteria and their byproducts. Till today, various dyes such as methylene blue, eosin, crystal violet, India ink, and Rhodamine B have been used[4] but in the present study, Rhodamine B dye was used as it is an organic acid compounded by a red-violet powder, classified as a xanthenic dye, presents greater diffusion on human dentin.

In our study, Confocal Laser Scanning Microscope (CLSM) examination was used to determine the sealing ability of root-end filling materials by measuring the depth of linear dye penetration as it has greater advantage over a conventional microscope because it rejects the light that does not come from the focal plane, enabling one to perform optical slicing and constriction of three-dimensional (3D) images. Further its high axial resolution, sharp image quality and associated quantitative image analysis provides vital structural information in the mesoscopic range for full 3D realization of the microstructure [17].

Thus, the results in our study are in consistence with many of the previous studies [18], however the minor variations can be attributed to small sample size selected, difference in the dye penetration method used and Confocal Laser Scanning microscopy technique adopted.

CONCLUSION

Hence, our study concludes that MTA remains to be the best retrofilling material showing minimal leakage and Bioaggregate showed better results than Intermediate Restorative Material. However, direct extrapolation of these results to clinical application requires further invitro and invivo studies, to evaluate Bioaggregate as a root end filling material.

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