

Comparative Evaluation of the Sealing Abilities of Different Materials as Barriers to Coronal and Apical Microleakage Using Dye Penetration Method-An In Vitro Study

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Abstract

Introduction: The main objective of root canal filling is to obturate the entire root canal system and provide an impervious apical and coronal seal. Incomplete obturation and sealing may cause penetration of microorganisms and toxins which is a major cause of endodontic failures.

Methodology: 90 recently extracted single rooted mandibular premolar teeth were collected for the study. The samples were randomly divided into 2 experimental Groups and 1 control Group, each involving 30 teeth. All the teeth were free from root defects, fractures and immature apices. All teeth were instrumented using Crown down technique with Hand NiTi Protaper files. The samples were immersed in 2% methylene blue dye for 7 days to allow dye penetration. All the samples were split longitudinally and visualized under Stereomicroscope at a magnification of 10X for measuring linear dye penetration.

Results: The data was analysed using **Student's 't' Test** at 5% level of significance and **'Z' Test** at 5% level of significance.

Conclusion: Present study concluded that Resin Composite and Resin Modified Glass Ionomer cement

present better sealing ability than dental amalgam when used as post endodontic coronal restoration.

Key words: Apicoectomy, Dye Penetration, Gutta percha, Lateral Condensation, MTA, Microleakage, Methylene Blue, Obturation, Resin Modified GIC, Stereomicroscope.

Introduction

Complete obliteration of the root canal space and development of fluid tight seal are among the most important factors for successful endodontic treatment¹. The main objective of root canal filling is to obturate the entire root canal system and provide an impervious apical and coronal seal. Incomplete obturation and sealing may cause penetration of microorganisms and toxins which is a major cause of endodontic failures. However, even with the best level of achievement, both coronal and periradicular, leakage of fluids into the canal system can occur²

Microleakage is defined as the clinically undetected passage of bacteria, fluids, molecules or ions between a cavity wall and the restorative material applied to it. These microorganisms play a crucial and critical role in pulpal and periapical diseases and thus in success and failure of endodontic treatment. Therefore, the role of apical and coronal sealing as a part of endodontic treatment cannot be underestimated.³

The most common cause for failure of root canal therapy is apical percolation or microleakage due to an inadequate apical seal¹⁵. This allows periapical fluids, proteins, and bacteria to gain access to the root canal system. Through this interchange, an inflammatory reaction is initiated which often results in radiographic or clinical signs of failure of root canal therapy. The question arises that if apical microleakage is a cause of endodontic failure, what role might coronal microleakage play in prognosis of root canal treatment? Endodontic obturation is often thought of only in terms of an effective apical seal. However, the coronal seal is equally important for the ultimate success of endodontic treatment.⁴

In addition to a good apical seal an intact coronal seal is important for successful endodontic therapy. A tooth filling material must exhibit good adherence, marginal adaptation, biocompatibility, reinforcement and longevity. To prevent microleakage it must be able to provide a long lasting seal against oral fluids and microorganisms⁶. No dental restorative material matches all the requirements of an ideal post endodontic restorative material.⁴ Various restorative materials such as Resin Composite, Amalgam, Bonded Amalgam, Glass Ionomer Cement and their modifications etc. have been advocated and used as post endodontic coronal restorative material.⁷ In situations where the canal space cannot be adequately sealed with conventional endodontic therapy, the canal may be sealed following surgical exposure by preparation of the root apex and placement of a retrofilling. Retrograde root filling following apicoectomy is a prudent procedure aimed at sealing the root canal to prevent the penetration of tissue fluids into the root canal or leakage of microorganisms and their toxins through the apical foramina into the surrounding tissues^{8, 9, 10, 11}. Various materials have been suggested for retrograde filling. An ideal retrograde filling material should adhere and adapt to

the dentinal walls of the root end preparations, should prevent leakage of microorganisms and their byproducts into the periapical tissues and should be biocompatible with the periapical tissues. It should also be non-toxic, non-carcinogenic, insoluble in tissue fluids, dimensionally stable, and not susceptible to the presence of moisture.^{12, 13}

Various dental restorative materials have been advocated as retro filling materials. These include gutta percha, zinc oxide eugenol paste, MTA, Ca phosphate cements, zinc phosphate, cavite, composite resins, gold foil, glass ionomers, IRM, super EBA, hydron, standard amalgams, cyanoacrylates etc.. None have met all the criteria set up in the literature.^{13, 14}

The present study was conducted to compare and evaluate the sealing abilities of different materials as barriers to coronal and apical microleakage and to determine the significance of coronal and apical microleakage as a source of endodontic failure.

AIM

The aim of the present study is to compare and evaluate the sealing ability of different materials as barrier to coronal and apical micro-leakage and determine their role in endodontic failures.

Objectives

1. To compare the amount of micro leakage among different materials used as coronal restorations following endodontic treatment.
2. To compare the amount of micro leakage among different materials used as apical (root end) restorations following endodontic treatment
3. To determine which of the two, coronal or apical micro-leakage is a major cause of endodontic failure.

Methodology

The present study was carried out in the Department of Conservative Dentistry and Endodontics, Subharti Dental

College, Meerut, in association with National Bureau of Plant Research and Genetics, Delhi

Selection of Samples:

90 recently extracted human mandibular premolars with single root canal were selected. The samples were cleaned and stored in normal saline solution till they were subjected to experimental study. All the teeth were free from caries, root defects, fractures, craze lines and immature apices. None of the selected teeth were previously root filled or had any restorations. The teeth were also monitored radiographically and those presenting radicular calcifications or anatomic abnormalities were excluded.

Access Cavity Preparation Chemo- Mechanical Preparation Obturation of the Samples Division of Groups

The prepared samples were then randomly divided into 3 groups of 30 teeth each as follows:

Group A- Apical micro leakage group (Retrograde restoration) (n=30)

This group consisted of 30 samples in which apical micro leakage study was carried out after apicoectomy and placing retrograde fillings using different materials. Each sample was circumferentially marked with a marker at 3mm from the apex. Apicoectomy was performed on each sample at the level of the marking at an angle of 90 degree to the long axis of the root using a flat fissure diamond bur in a high speed handpiece using air water coolant. Care was taken to avoid dragging of obturated material along with bur. The resected end was inspected for smoothness. A retrograde class I cavity was cut along the long axis of each tooth with inverted-cone bur in a slow speed hand piece to a depth of 3mm (marked on the bur) at the apical end (resected root surface) of each sample .

The 30 sample teeth were then randomly divided into three sub-groups of 10 samples each according to the different retro-filling materials.

Sub- group A_A-Retro cavities of all samples in this subgroup were filled with High copper amalgam (n=10)

Sub- group A_G- Retro cavities of all samples in this subgroup were filled with Resin modified GIC (n=10)

Sub- group A_M- Retro cavities of all samples in this subgroup were filled with Pro Root Mineral Trioxide Aggregate (n=10)

The materials were manipulated and placed according to manufacturer's directions and radiographs were taken to assess the completeness of retrograde fillings.

Sub- group A_A-High Copper Amalgam alloy was triturated with mercury in mortar with a pestle and muller in chamois according to manufacturer's recommendation. With the help of amalgam carrier the mixed alloy was placed into retro- cavities of the specimens, condensed with a serrated round condenser in increments until at level with the preparation surface and burnished with ball burnisher after the initial set.

Sub- group A_G- GC Fuji II LC was mixed according to manufacturer's recommendation. The retro cavity was rinsed thoroughly with water and blotted dry. Precautions were taken not to desiccate the surface. The powder and liquid were dispensed on the mixing pad. Gradually the cement was incorporated in the liquid and mixed for about 20-25 seconds. The mixed material was carried with the help of a plastic carrying instrument, and placed into retro-cavities of the samples and condensed using plastic instrument until at level with the preparation surface and light cured for 20 seconds.

Sub- group A_M- Pro Root Mineral Trioxide Aggregate was mixed using supplied water packets (according to manufacturer's recommendation).The powder and liquid were dispensed on the mixing pad and gradually the liquid

was incorporated in the cement using plastic spatula. The material was mixed with liquid for about a minute to ensure that all the powder particles are hydrated. The mixed material was then placed incrementally with messing gun into the apical preparation and condensed until at level with the preparation surface.

After root-end filling was done in all the samples, two coats of nail varnish were applied on the whole tooth except the root-end prepared portion (resected root surface) before immersing in the dye. The second coat was applied only after the first coat had dried.

Group B-Control group (n=30)

Two coats of nail varnish were applied on the whole tooth while leaving the apical (5mm) portion before immersing in the dye.

Group C- Coronal micro leakage group (Coronal restoration) (n=30)

This group consisted of 30 samples in which coronal microleakage study was carried out for different permanent restorative materials used to seal the access cavities. Cavit-G was removed from all the samples leaving behind 1mm of Cavit placed over the obturating material to serve as base for permanent restorative material. Cavit-G was removed using inverted cone and straight fissure bur in high speed airtor handpiece to a depth of 3mm below the margins of access cavity. A probe was used to control the thickness of the restorative materials. The cavities were flushed with water and the walls were inspected for any traces of Cavit-G to be removed.

30 sample teeth of this group were further subdivided into 3 sub groups of 10 samples each (n=10) according to the restorative material used to seal/restore the access cavities.

Sub-group C_A-Access cavities of samples in this group were restored with High copper amalgam (DPI,Alloy Fine Grain, Mumbai) (n=10)

Sub- group C_G- Access cavities of samples in this group were restored with Resin modified GIC (GC Fuji II LC) (n=10)

Sub- group C_R- Access cavities of samples in this group were restored with Composite resin (Filtek Z 250) (3M ESPE) (n=10)

The materials were manipulated and placed according to manufacturer's directions.

Sub-group C_A- High copper amalgam alloy was triturated with mercury in mortar with pestle and muller in chamois according to manufacturer's recommendation. With the help of amalgam carrier the mixed alloy was placed into access cavity of the samples and condensed with a serrated round condenser in increments until whole of the cavity was completely filled. Each restoration was burnished with ball burnisher after the initial set.

Sub- group C_G- The access cavity was rinsed thoroughly with water and blotted dry. Precautions were taken not to desiccate the surface. The powder and liquid were dispensed on the mixing pad and gradually the cement was incorporated in the liquid and mixed for about 20-25 seconds. The mixed material was placed into the cavity using plastic carrier instrument, condensed using plastic instrument and light cured for 20 seconds.

Sub- group C_R- The access cavity of samples in this group was etched for 15 seconds using 37% phosphoric acid as etchant. The etchant was applied with the help of applicator tips. The etchant was washed off from the cavity with distilled water for 10 seconds, and gently blotted dry. Precautions were taken not to desiccate the surface. A thin layer of adhesive was applied with applicator tips and light cured for 10 seconds. Access cavity was then restored with Resin Composite using incremental technique. Each increment was placed into the cavity using plastic carrier instrument, condensed and cured for 20 seconds. The process was repeated until

whole of the cavity was completely filled. Each restoration was then polished using composite polishing stones.

After completion of coronal restoration in all the samples, two coats of nail varnish were applied on the whole of the root surface from CEJ to the root apex, leaving the crown portion uncoated /uncovered.

Dye Immersion

All the samples of the three groups (Gp A, GpB,Gp C)were then subjected to microleakage study using dye penetration method . The samples were immersed in 2% methylene blue dye and were left undisturbed for 7 days to allow dye penetration.

Microleakage Study

After 7 days the samples were removed from dye, washed carefully under tap and allowed to dry. All the samples were carefully spilt buccolingually into two longitudinal halves. A double sided diamond disc in a slow speed straight hand piece was used to make two opposing deep longitudinal grooves buccally and lingually along the entire length of the tooth. The samples teeth were then spilt into two halves by using chisel and mallet. Care was taken to avoid loss or fracture of the sample teeth.

The restorations (both apical as well as coronal) were removed from the respective halves of all the samples using round bur and ultrasonic scaler.

Finally the spilt halves of all the samples were visualized under Stereomicroscope at a magnification of 10X for measuring linear dye penetration using a graph paper.

Observaion and Result

The present study was conducted to compare the sealing abilities of different materials used as post endodontic coronal and retrograde restorations and determine the significance of coronal and apical microleakage as a source of endodontic failure.

The obtained values and results were evaluated using tables and bar diagrams.

Table 1.Shows the values of linear dye penetration in each sample of all the sub-groups of Group A (Apical Group/ Retrograde restoration Group).It also summarizes, the mean value and the standard deviation in each sub-group.

SAMPLE NO.	Dye Penetration (in mm)		
	SUB-GROUP A _A (AMALGAM RETROFILLINGS)	SUB-GROUP A _G (GIC RETROFILLINGS)	SUB-GROUP A _M (MTA RETROFILLINGS)
1.	2.0	2.4	1.2
2.	3.0	2.2	1.0
3.	2.0	2.1	1.5
4.	1.5	1.0	0.8
5.	2.5	0.5	0.8
6.	2.5	0.2	0.5
7.	2.4	0.8	1.0
8.	3.0	1.0	2.0
9.	1.5	0.8	1.0
10.	2.0	0.8	1.0
MEAN	$\Sigma 22.4/10=2.24$	$\Sigma 11.8/10=1.18$	$\Sigma 10.8/10=1.08$
STANDARD DEVIATION	± 0.845308	± 0.83006	± 0.415799

From the above analysis it is clear that **sub-group A_A** (Amalgam restoration) showed maximum microleakage whereas, **sub-group A_M** (MTA restoration) showed minimum microleakage.

Further **Student’s’t’Test** was applied to test the significance difference for intra group comparison in the microleakage scores of the 3 sub-groups of Group A **at 5% level of significance.**

Table -2 shows the statistical analysis of intra group comparison.of Group A.The comparison was made by applying **Student’s 't' Test** statistic and obtaining't' value.

S.No.	Sub-Groups Under Comparison	Mean	SD	t _{calculated}	t _{tabulated} (.05,18)
1.	AMALGAM(A _A) and GIC(A _C)	2.24 and 1.18	±0.83006 and ±0.845308	3.583	2.10
				t _{cal>t_{tab}}	
2.	GIC(A _C) and MTA(A _M)	1.18 and 1.08	±0.845308 and ±0.415799	0.3624	2.10
				t _{cal<t_{tab}}	
3.	AMALGAM(A _A) and MTA(A _M)	2.24 and 1.08	±0.845308 and ±0.415799	5.41	2.10
				t _{cal>t_{tab}}	

Table 3. Shows the values of linear dye penetration in each sample of all the sub-groups of Group C (Coronal Group/ Coronal Restoration Group). It also summarizes the mean value and the standard deviation in each sub-group.

SAMPLE NO.	Dye Penetration (in mm)		
	SUB-GROUP C _A (AMALGAM RESTORATION)	SUB-GROUP C _G (GIC RESTORATION)	SUB-GROUP C _R (COMPOSITE RESTORATION)
1.	4.0	1.2	0.8
2.	4.0	2.2	1.2
3.	2.5	0.8	1.0
4.	2.8	0.8	1.5
5.	4.0	1.0	1.0
6.	3.2	0.9	1.2
7.	3.0	2.1	1.0
8.	2.6	1.5	0.8
9.	3.1	1.4	1.5
10.	3.4	1.1	1.4
MEAN	∑32.6/10=3.26	∑13/10=1.3	∑11.4/10=1.14
STANDARD DEVIATION	±1.12452	± 0.649786	± 0.263312

Further **Student's 't' Test** was applied to test the significance difference between for intra group comparison in the microleakage scores of the 3 sub-groups of Group C at **5% level of significance**.

Table -4 shows the statistical analysis of intra group comparison. The comparison was made by applying **Student's 't' Test** statistic and obtaining 't' value.

S.No.	Sub-Groups Under Comparison	Mean	SD	t _{calculated}	t _{tabulated} (.05,18)
1.	AMALGAM(C _A) and GIC(C _C)	3.26 and 1.3	±1.12452 and ±0.649786	14.9269	2.10
				t _{cal>t_{tab}}	
2.	GIC(C _C) and COMPOSITE (C _R)	1.3 and 1.14	±0.649786 and ±0.263312	0.8877	2.10
				t _{cal<t_{tab}}	
3.	AMALGAM(C _A) and COMPOSITE (C _R)	3.26 and 1.14	±1.12452 and ±0.263312	10.59	2.10
				t _{cal>t_{tab}}	

Table 5. Shows the values of linear dye penetration in each sample of **Group B** (Control Group). It also summarizes the mean value and the standard deviation of the group. Minimum individual microleakage score was observed in samples 28 & 29 of **Group Bi.e 2.0** Maximum individual microleakage score was observed in sample 6 of **Group Bi.e 11**.

Sample No.	Dye Penetration(in mm)
	Control Group
1.	10
2.	8
3.	3
4.	4
5.	5
6.	11
7.	9
8.	3

9.	4
10.	5
11.	6
12.	4
13.	3
14.	6
15.	7
16.	3
17.	5
18.	4
19.	3
20.	4
21.	10
22.	6
23.	3
24.	3
25.	6
26.	4
27.	3
28.	2
29.	2
30.	4
MEAN	$\sum 150/30=5$

STAND ARD DEVIAT ION	± 2.46352

Thereafter 'Z' Test was applied to the obtained readings in order to find whether or not statistically significant difference (at 5% level of significance) in values of dye penetration among the 3 Groups. The results of Z test are presented in Table 7.

Table -6 shows the statistical analysis of inter group comparison. The comparison was made by applying 'Z' test statistic and obtaining 'Z' value.

It can be concluded that:

- 1) Statistically significant difference was observed between microleakage scores of Group C &B, Group B& A ($z_{cal} > z_{tab}$)
- 2) No statistically significant difference was observed between microleakage scores of Group C and A ($z_{cal} < z_{tab}$)

In the Bar diagram1, vertical line 'a' depicts statistically significant difference between Sub-Group A_G (GIC) and A_A (AMALGAM).

Vertical line 'b' depicts, statistically significant difference between Sub-Group A_M (MTA) and A_A (AMALGAM).

Star 'c' depicts, no statistically significant difference between Sub-Group A_M (MTA) and A_G (GIC)

In the Bar diagram 2, vertical line 'a' depicts a statistically significant difference between Sub-Group C_G (GIC) and C_A (AMALGAM).

Vertical line 'b' depicts statistically significant difference between Sub-Group C_R (COMPOSITE) and C_A (AMALGAM).

Star 'c' depicts, no statistically significant difference between Sub-Group C_R (COMPOSITE) and C_G (GIC)

Results

On completion of the study and after subjecting the obtained values to statistical analysis it was observed that:-

(a) Microleakage in samples with coronal resin composite (Filtek Z250) and resin modified GIC (GC Fuji II LC) restorations was significantly lesser than in samples with coronal amalgam restorations.

(b) Microleakage in samples with retrofilled Pro Root MTA (DENTSPLY) and resin modified GIC (GC Fuji II LC) restorations was significantly lesser than in samples with retrofilled amalgam restorations.

(c) Apical microleakage in samples without any retrofilling was significantly greater than coronal microleakage in samples with coronal restorations and apical microleakage in samples with retrograde restorations.

Discussion

Clinical endodontics encompasses a number of treatments but perhaps the most important is treating pulps and root canal systems with or without periradicular pathosis of pulpal origin rendering the patient to retain their natural teeth in function and esthetics.¹

When dental pulp undergoes pathological changes because of trauma or the progression of dental caries, bacteria and other irritants from the oral cavity invade the root canal system. The major objective of root canal therapy is the removal of pathologic pulp, cleaning, and shaping of the root canal system, disinfection of contaminated root canals, followed by the three-dimensional obturation and post endodontic restoration to prevent reinfection².

Once a fluid-tight seal is established, any inflammatory reaction initiated or promoted by noxious material from within the canal should cease. This allows healing of periradicular pathosis. However, if the canal is not completely cleaned and sealed (both apically and coronally) inflammation and infection may continue. Obturation is a reflection of cleaning and shaping and is necessary to eliminate leakage. It prevents apical and coronal leakage, bacterial contamination, seals the apex from the periapical tissue, and seals the remaining irritants in the canal¹⁶

Microleakage at the tooth/ restoration interface is considered to be a major factor influencing the longevity of dental restorations and the outcome of the endodontic treatment, as it may lead to development of periapical pathology. It has been known for many years that conventional restorative materials and techniques produce dental restorations that do not provide complete marginal seal, and numerous studies have demonstrated that leakage of fluid will occur between the filling and the prepared tooth surface. Micro organisms play a crucial and critical role in pulpal and periapical diseases and thus in success and failure of endodontic treatment.³

Coronal leakage has been indicated in the literature as one of the major determinant of endodontic success or failure.^{17, 18} Coronal leakage provides a constant source of microorganisms and nutrients that initiate and maintain periradicular inflammation and may well be the largest cause of failure in endodontic therapy¹⁹. If the coronal portion of the tooth is not sealed with materials that bond to tooth structure and are resistant to dissolution by oral fluids, then, over time endodontic failure may be inevitable. Coronal leakage for even a minimal amount of time may quickly lead to apical migration of bacteria. In this respect the importance of perfectly sealing coronal restorations (both temporary and permanent) needs to be

emphasized¹². Some of the causes for loss of coronal seal after endodontic treatment are delay in placing a restoration, fracture of restoration, or post space preparation when remaining apical section of root filling is inadequate²⁰

Post endodontic restorative material should prevent root canal system contamination from oral fluids and food debris. To meet these criteria, sealing ability is the prime consideration in selecting a permanent restorative material after endodontic treatment.⁵

A restorative material must exhibit good adherence and adaptability to the walls of the prepared cavity. It must be compatible with the surrounding tissue, and must not cause staining or other adverse effects to the surrounding tooth structure. It must harden to provide structural support for the tooth, including its biting surface. It must be relatively easy to apply in what is sometimes a difficult environment due to blood, moisture and potential access problems. It is also important for diagnostic purposes that a filling material be radiopaque. It should also be sterile or easily sterilized.^{6, 21}

However, no dental restorative material matches all the requirements of an ideal post endodontic restorative material.⁴ Various restorative materials have been advocated and used as post endodontic coronal restorative material. These materials include Resin Composite, Amalgam, Bonded Amalgam, Glass Ionomer Cement and their modifications etc.⁷

Development of modern composite restorative materials started in late 1950's and early 1960's. Dental resin composites are highly cross linked polymeric material reinforced by dispersion of glass, crystalline or resin filler particles and /or short fibres bound to the matrix by silane coupling agents. These composite materials have inherent advantage of being tooth coloured and are micro mechanically bonded to the tooth surface. When used as a

permanent restorative material, these materials allow conservation of tooth structure while simultaneously resulting in good retention, low microleakage, minimal interfacial staining and reinforcement of remaining tooth structure. Composites have been indicated for use in sealing the coronal access cavity after endodontic therapy as they provide good marginal sealing ability and good esthetics than other materials¹⁵.

Glass ionomer cements were developed in late 1960's. They are tooth coloured and adhere chemically to tooth tissue. To overcome the disadvantages of conventional glass ionomer cements such as moisture sensitivity, susceptibility to desiccation and to widen the range of clinical applications, *Mathis arid Fetracane (1989)* introduced the resin modified glass ionomer cement. A resin component is incorporated into the cement and is cured with visible light, which gives the advantage of longer working time and better bond strength, combined with chemical bonding and fluoride releasing. The RMGIC is defined as a material which undergoes both polymerization reaction and an acid-base reaction. Properties similar to conventional glass ionomer, makes it a good option for sealing the coronal access cavity in root canal treated teeth.²²

In addition to coronal seal, apical seal is equally important to prevent microleakage after endodontic treatment.

In situations where an adequate apical seal cannot be achieved with conventional endodontic therapy, the canal may be sealed following surgical exposure by preparation of the root apex and placement of a retrofilling. Apicoectomy involves resection of the root tip to eliminate the aberrant anatomic entities and filling the resected root with the suitable root end filling material. This provides an apical seal to prevent the microleakage through the interface of tooth and restoration. It will also prevent periradicular tissue fluid infiltration. With this

purpose of obtaining root end sealing, various retrograde filling materials have been used in the past.^{9,10,11}

According to Gartner and Dorn an ideal retrograde filling material should prevent leakage of microorganisms and their by products into periradicular tissues. It should be well tolerated by periapical tissues, adhere to tooth structure, should be dimensionally stable, resistant to dissolution and should promote cementogenesis. It should be bacteriocidal or bacteriostatic, non corrosive and electrochemically inactive. It should not stain tooth structure or periradicular tissues. It should allow adequate working time and a short setting time. Finally it should be radiopaque.^{53,54}

The materials which have been suggested as retro filling materials include zinc oxide eugenol paste, MTA, Ca phosphate cements, zinc phosphate, Cavit, resin composite, gold foil, glass ionomers, IRM, Super EBA, Hydron, standard amalgams, cyanoacrylates etc. The suitability of these materials as retrograde filling materials has been tested by their sealing ability, marginal adaptation to the dentinal walls, biocompatibility and their clinical performance. None have met all the criteria set up in the literature.^{13, 14, 54}

In the present study Amalgam, Resin modified GIC and MTA were used as retrograde filling materials.

Dental amalgam has been a standard for comparison with other restorative materials. Use of dental amalgam as a retrograde filling material has been advocated, as it is easy to manipulate, good radio opacity, non soluble in tissue, and marginal adaptation as well as sealing improves as amalgam ages, but has few limitation which include initial marginal leakage, delayed expansion, tissue staining, corrosion, tin and mercury contamination of periapical tissue.^{14,31,54}

Another material, Resin modified GIC (which was introduced to overcome the shortcomings of the

conventional GIC'S) has also been used as retrograde filling material. It provides a superior sealing ability because of its chemical adherence to dentin, and light curing also enables command setting, although the material will set in absence of light. The high density of the material and the chemical fusion between tooth tissue and GIC results in decreased leakage of microorganism and their toxins when it is used as retrograde filling material. It has the desirable properties of good seal, relatively easy mixing and placement, contrasting color to tooth structure, biocompatibility and short setting time.^{22, 27}

Mineral Trioxide Aggregate (MTA) introduced in 1993 by *Torabinejad* has become very popular and widely used as a root end filling material. It contains Tricalcium silicate, Tricalcium Aluminate. Tricalcium oxide. Silicate oxide and other mineral oxides forming a hydrophilic powder which sets in the presence of water. It provides superior seal when compared with other materials. MTA has also shown evidence of healing of surrounding tissue when used as Retrograde Filling Material. Moreover its property of inducing hard tissue formation and not being affected by blood contamination has made it a choice of material for Retrograde Filling.^{12,31}

The present study was thus conducted to compare and evaluate the sealing abilities of different materials as barriers to coronal and apical microleakage and to assess whether coronal or apical microleakage is a major source of endodontic failure.

In the present study, 90 freshly extracted human mandibular premolars with single root canal were selected. All the teeth were free from caries, root defects, fractures, craze lines and immature apices. The similar criteria for sample selection have been followed in other studies^{3, 6, 24, 16} on microleakage analysis.

The samples were cleaned and stored in normal physiologic saline solution till they were subjected to experimental study. They were stored in physiologic saline to prevent dehydration of dentin and to simulate in vivo conditions. Saline has been used as an acceptable preservative medium for storage as well as an effective disinfecting agent.⁷⁰

Access cavity preparation was done using the same bur size for all the samples to standardize the shape and size of access cavity.

The working length in this study was determined by viewing the No. 10 K-file instrument tip at the apex and then retracting it 1 mm short of apex. Similar technique for working length determination has been mentioned in other studies.^{7, 69, 63}

The prepared samples were then randomly divided into 3 groups of 30 teeth each as follows:

Group A- Apical micro leakage group (Retrograde restoration) (n=30)

Apicoectomy was performed in all samples of this group. Each root was resected perpendicular to long axis of root at 3 mm from the apex. This helps in conserving tooth structure thereby improving crown /root ratio while meeting the objective of removing the vast majority of apical ramifications. It also leads to lesser exposure of apical surface and dentinal tubules thereby decreasing the microleakage. In all the samples Class I retrograde cavities were then prepared^{9, 13, 78}

Further the samples in the group were divided into 3 subgroups and accordingly the retrograde cavities were restored with the 3 retrograde filling materials used in the study (Amalgam, RMGIC and Pro Root MTA). All the samples were then coated with 2 layers of nail varnish leaving the resected end.

Group B-Control group (n=30)

Samples in this group were not subjected to any root end preparation and restoration in order to simulate the microleakage occurring in vivo in conventional root canal treatment. The samples were coated with 2 layers of nail varnish except for the Apical 5 mm.

Group C- Coronal micro leakage group (Coronal restoration) (n=30)

In this group, access cavities were restored with the 3 post endodontic restorative materials used in the study (Amalgam, Resin Composite and RMGIC). All the samples were then coated with 2 layers of nail varnish leaving the coronal portion till the CEJ.

Different methods have been used in literature for invitro assessment of microleakage of restorations. These include the use of dyes, chemical tracers, radioactive isotopes, air pressure, bacteria, neutron activation analysis, scanning electron microscopy, artificial caries techniques and electrical conductivity. Dye penetration method was selected as it is easy, detectable in dilute concentrations, inexpensive and nontoxic³. This method for microleakage detection has been used extensively and reliably in other studies.^{63, 49}

The samples were immersed in 2% methylene dye and kept in incubator at 37°C to simulate in vivo environment. Methylene blue dye has been chosen for the study because of its low molecular weight that facilitates its diffusion. The size of the dye particle is smaller than the size of bacteria which confirms to its highly sensitive nature. Another advantage of using this dye is that, its pH (7.4) simulates the pH of blood which helps in mimicking the in vivo conditions⁷¹. The use of Methylene blue dye for microleakage assessment has also been supported in studies.^{63, 49}

The samples were immediately immersed in the dye after coronal and retrograde restorations because all the materials tend to leak more during initial setting period.

This may be due the shrinkage of most of the material during their setting. Dehydration at the interface of the material and dentine during the setting process of the material causes the dehydrated area to soak up more of the dye.²⁵

After removing from dye, the samples were washed carefully under tap water and allowed to dry. The samples were carefully spilt buccolingually into two longitudinal halves. The restorations (both apical as well as coronal) were removed from the respective halves of all the samples using round bur and ultrasonic scaler, before the spilt halves were subjected to Stereomicroscopic analysis¹³ at 10x magnification⁶³.

Sectioning reveals significantly higher dye penetration values than the clearing technique⁷⁸. Stereomicroscope at 10x magnification was used for the present study because thicker sections of the samples could be easily visualized under this microscope.

In the present study all the samples of Group A (Apical Group) showed microleakage. Samples retro- filled with Pro Root MTA (Sub group A_M) showed minimum microleakage when compared to samples retro-filled with Resin modified GIC (Sub group A_G) and Amalgam (Sub group A_A). Similar results have been reported in other studies.^{22, 25, 29, 34}

Pro Root MTA was introduced as a new root end filling material at Loma Linda University, California, USA in 1993 by Torabinejad. Unlike a number of dental materials that are not moisture tolerant, Pro Root MTA actually requires moisture to set. This results in expansion of MTA during its setting in moist environment, which in turn increases the marginal adaptation.^{34,45} Pro Root MTA has been shown to demonstrate marginal gaps ranging from 0.5 -1 µm wide (much less than other materials). Therefore, the better sealing ability of Pro Root MTA is due to its better marginal adaptation to dentinal wall.

The sealing ability of MTA was investigated by various methods other than dye penetration. They all reported good results with MTA when ranked with other materials. This may be because of its moisture tolerance and long setting time which favors its use as a retro filling material.⁷²

MTA also encourages hard tissue deposition, a unique phenomenon, not been reported in other root end filling materials. It induces cementogenesis and offers a biologically active substrate for osteoblasts, while also stimulating cytokines required for the formation of bone.^{39,23,81}

MTA particles have a small size (1.5µm) which makes it possible for them to enter into open dentinal tubules (2.5µm). This might be an important mechanism to provide a hydraulic seal⁷³

In the Apical Group, samples retro filled with GC Fuji II LC (RMGIC) showed more microleakage than Pro Root MTA retro fillings. GC Fuji II LC (RMGIC), however showed significantly less microleakage than retrofilled Amalgam restorations. This in corroboration with other studies.^{13, 15, 22, 30, 18, 31, 29}

RMGIC has the properties of conventional GIC with an added advantage of faster setting time and micromechanical bonding. Their setting mechanism is defined by the term 'dual cure' which implies both free radical polymerization and acid-base reaction to set into a hard mass. They have good adhesion to dentin and light curing also commands setting, although the material will set in absence of light. Various studies support the use of RMGIC as a retrograde sealing material.^{30,11,12,36,27,29.}

It has been shown that thinly applied (>1mm) RMGIC retroseal permitted less microleakage when compared with thicker fillings. Also, that RMGIC when directly smeared over the resected root surface without any retrograde

cavity provided a better adaptation to the resected root surface.²²

The greater microleakage seen with RMGIC when compared with Pro Root MTA can be explained by the fact that in this study RMGIC was applied in thickness of 3mm. This was done to ensure similarity in all sub-groups. RMGIC also undergoes polymerization shrinkage. It has been observed that in deep cavities it is well adapted to one of the cavity walls but gaps are present on opposite cavity walls as a result of contraction stresses.²²

Another possible explanation can be due to development of surface cracks as a result of polymerization contraction when RMGIC is used in thicker bulk (>1mm).⁹² Proponents of RMGIC as a retrograde filling material suggest the use of RMGIC without any retrograde cavity preparation.²² This also makes RMGIC as a choice of retrograde filling material in areas where access is limited in a surgical field.

Although Resin modified GIC's are easier to handle compared to conventional GIC but the need for total dryness before placement is questionable. Even when a dry field is maintained, some moisture is inevitable and this may affect the glass ionomer / dentine bond which in turn affects its sealing ability as a retrograde restorative material.²⁹

In this study RMGIC shows better results than Amalgam because of its chemical adhesion to the tooth structure, thereby forming a strong, long lasting physiochemical bond whereas Amalgam is not truly adhesive and requires mechanical retention.^{22, 29, 31} Further RMGIC is hydrophilic in nature which may be advantageous when used in aqueous biological system as compared to hydrophobic nature of Amalgam.⁷⁶

All the samples of the Amalgam in the present study showed maximum leakage because of its poor marginal adaptability to the tooth structure. The marginal adaptation

of the retrograde fillings is assumed to reflect their sealing potential. Amalgam has been shown to demonstrate marginal gaps ranging from 6-150µm wide which could be a reason for their clinical failure in relation to its poor sealing ability. It does not adhere to the tooth structure and also contracts during its initial setting, which leads to microleakage. Corrosion products which tend to seal the tooth/restoration interface may take over 6 months to develop in-vivo thus reducing microleakage³⁹ but this long period of observation is not suitable for microleakage study as microleakage under in-vivo conditions starts as soon as the restorative material is placed. The results of the present study correspond to the earlier studies done where Amalgam showed maximum dye leakage.^{19, 31}

Even when performed under the best conditions and with suitable materials, retrograde filling of the root canal cannot be considered a substitute for a thorough treatment of the entire root canal. Because success in nonsurgical endodontics is based on the principles of thorough debridement and complete obturation of the root canal system, it is logical not to ignore or compromise these principles for teeth requiring endodontic surgery.¹¹

All the samples in Control Group (Group B) showed severe microleakage which was significantly more when compared to the coronal restoration group (Group C) and retrograde restoration (Group A).

Control Group was used in the study to evaluate the amount of microleakage after conventional endodontic therapy and to observe whether apicoectomy followed by retrograde restoration has any significant effect on reducing microleakage.

It can be argued that a retro filling may be avoided if radiographically the obturation appears to be well condensed but this argument is not well supported since studies have

shown that apparently well obturated canals may be infected.¹¹

Various other microleakage studies suggest that microleakage does occur after conventional endodontic treatment and radiographic adequacy of obturation cannot be taken as single most criteria for producing a fluid-tight apical seal as radiographs present a two dimensional view of the three dimensional system.¹¹

Since microleakage was significantly reduced after apicoectomy and retrograde restoration as has been suggested by other studies^{6, 42} therefore, the significance of inclusion of Control Group in this study is justified.

In the coronal group microleakage was observed in all the samples with coronal restoration. This is in accordance with results of other studies which suggest that none of the various post endodontic coronal restorative materials is able to prevent microleakage completely.

In the present study all the samples coronally restored with Resins Composites (Filtek 250, Subgroup C_R) and RMGIC (GC Fuji II LC, Subgroup C_G) showed significantly less microleakage than samples restored with Amalgam restoration (Subgroup C_A). This is in accordance with results of other studies¹⁵

These results are in accordance with other studies in which coronal amalgam restorations were found to leak more as compared to composite and RMGIC restoration.

In the coronal group samples coronally restored with Composites Resins (Subgroup C_R) showed minimum microleakage.

Resins Composite, has been used in dentistry for more than a decade and are most common choice for restoring access cavities⁷. Introduced by Bowen (1962) they are a compound of two or more distinctly different materials with properties that are superior to or intermediate to that of individual components. Since their introduction several development and improvements have been made in their

physical and mechanical properties which permitted their application in tooth restoration. They have shown good sealing ability, they bond to tooth structure and can provide a good match of color and surface gloss¹⁵. Bonded composite materials can strengthen existing coronal or radicular tooth structure, especially when used with newer generation bonding agents.

Better bonding, good marginal adaptation and decrease in microleakage can be attributed to the formation of resin tags and hybrid layer resulting in micro mechanical retention.¹⁸

Various studies have proved that the adhesion to the underlying tooth structure i.e. enamel and dentin can be increased when they are used in combination with Dentin Bonding Agents (DBA) with or without acid etching. The fifth generation DBA (Adper Single Bond) used in the present study necessitates the use of acid etching technique as it removes smear layer, widens dentinal tubules and causes demineralization of intertubular dentin as to create resinous tags. The primer in the bonding agent wets the collagen network resulting in the formation of hybrid layer.¹⁵

In the study Composite restorations was done using an incremental technique in which small increments of the resin composites resin were placed and cured before the addition of the next increment. This method of restoration build-up has shown to reduce microleakage by reducing polymerization shrinkage and contraction stresses¹⁵ thereby preventing marginal debonding. The composites resin Filtek Z 250 used in the study is a hybrid composite with microfiller (particles size 0.01-3.5µm) of silica /zirconia in a high volume % of 60%, such small particle size and high volume % of filler particles can also be advocated as a cause of reduced polymerization shrinkage and hence decreasing microleakage as seen in the present study.

Resin Composite also undergoes water sorption over a period of time thus negating the effects of contraction stresses. Thus, preventing marginal debonding and microleakage. However, this is a gradual process which develops over time. This may also be a contributory factor to reduced microleakage observed with Composite resins.²⁴

The relatively small amount of microleakage as observed with Composite resins may be attributed to the effects of polymerization shrinkage resulting in contraction stresses thus leading to marginal debonding and microleakage^{24, 15}.

Samples of the coronal group restored with RMGIC (Subgroup C_G) showed significantly less microleakage compared to amalgam restorations. This is in accordance with other studies where post endodontic coronal restoration of RMGIC provided better sealing ability than dental amalgam restorations^{9,10,14,80}

In the coronal group RMGIC showed significantly more microleakage as compared to composite resin restorations. This also well supported by other studies^{39,22}. Initial polymerization shrinkage associated with a greater bulk of material ($\approx 3\text{mm}$), as discussed earlier, accounts for increased marginal gap formation and crazing of enamel as compared to composite resin restorations.

Samples of the coronal group restored with dental amalgam (Subgroup C_A) showed maximum microleakage. Factors contributing to increased microleakage associated with coronal amalgam restorations can be related to lack of adhesive bonding and poor marginal adaptation of amalgam restorations⁸⁰. Poor marginal adaptation of dental amalgam can be attributed to its high creep rate as compared to other two restorative materials.⁵⁹ Lack of self sealing ability with high copper dental alloys can also be one of the contributing factors for the increased microleakage in amalgam.

Dental amalgam has a linear coefficient of thermal expansion 2.5 times greater than the tooth structure, this, coupled with the fact that it does not bond to the tooth structure can result in percolation of the fluids along the restoration tooth interface.⁸

Within the confines of the present study there was no statistically significant difference in the microleakage through coronal restorations (Group C) and through retrograde restorations (Group A) i.e. either the coronal or apical microleakage cannot be stressed upon as a more significant source of microleakage leading to failure of endodontic therapy.

Various researchers have investigated and implicated the role of coronal and apical microleakage in endodontic failure. However, we could not come upon any such study which compares coronal and apical microleakage simultaneously. Further studies need to be carried out using different restorative materials and microleakage detection techniques to provide a conclusive evidence if and which of the two is more responsible for endodontic failures. The findings of this study represents the results achieved in-vitro conditions. Various other static and dynamic factors are involved in the oral cavity the effect of which on microleakage needs to be tested further.

Conclusion

Within the limitations of the present study it can be concluded that:

1. Resin Composite (Filtek Z250) and Resin Modified Glass Ionomer cement (GC Fuji II LC) restorations show better sealing ability than High Copper Dental Amalgam (DPI), when used as post endodontic coronal restorations.
2. Pro Root MTA (DENTSPLY) and Resin Modified Glass Ionomer cement (GC Fuji II LC) restorations show better sealing ability than High Copper Dental Amalgam (DPI) when used as retro grade restorations.

3. Retro filling after apicoectomy reduces microleakage when compared to conventional endodontic treatment without retrofilling.

4. Any one of either coronal or apical microleakage could not be implicated as a more significant cause of endodontic failure.

However the results of the present study are bound to be variable under different circumstances with different materials and techniques. Therefore further research using different samples, a larger sample size and different material and method are needed to further substantiate the findings of the present study.

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