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A Comparative Evaluation of the Apical Microleakage in Root Canals Sealed with Bioceramic, Resin Based and Glass Ionomer Root Canal Sealers: An Ex-Vivo Study

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ABSTRACT

Background: Endodontic therapy consists of cleaning and shaping the root canal system, removing organic debris and sealing the intracanal space with permanent filling materials. The obturation must seal the pulp space both apically and laterally, thus preventing further apical irritation from either incomplete elimination of bacteria and their products or communication between apical tissues and oral cavity.

Aim & Objectives The purpose of this study was to evaluate and compare microleakage of Endosequence, RoekoSeal, Glass ionomer cement and Zinc oxide eugenol sealer when used as root canal sealer.

Method Leakage was assessed using a stereomicroscopic study with methylene blue dye as the medium. The cleared teeth were analysed by means of a steriomicroscope. The extent of dye penetration was measured in millimetres in all aspects of the root canal from the apical stop to the maximum length of dye penetration.

Conclusion The results were analysed statistically. There was significantly less leakage seen with RoekoSeal when compared to Endosequence, Glass ionomer cement and Zinc oxide eugenol sealer. Data were analysed statistically using Kruskal-Wallis multiple comparison test. Multiple comparisons were performed using the Mann-Whitney test.

Keywords: Microleakage, Sealers, Bio-ceramics, Endodontics, Root-canal.

INTRODUCTION

Comprehensive Debridement of the complexities of root canal system, followed by a fluid tight obturation of the prepared space in three dimensions is the foundation of successful endodontic treatment. The Washington study of endodontic success and failure has suggested that nearly 60% of failures are due to incomplete obturation of the root canal. Therefore, the goal of the root canal obturation is to create a three-dimensional fluid tight seal from the orifice to the apex with a stable, biologically inert material.

The most common causes of failure involving endodontic therapy can be attributed to the lack of apical seal leading to leakage at the apex. Effective endodontic obturation thus, must provide a dimensionally stable, inert and fluid tight seal that will eliminate any portal of communication between the canal space and the surrounding periapical tissues through apical foramen.

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Endodontic therapy consists of cleaning and shaping the root canal system, removing organic debris and sealing the intracanal space with permanent filling materials. The obturation must seal the pulp space both apically and laterally, thus preventing further apical irritation from either incomplete elimination of bacteria and their products or communication between apical tissues and oral cavity.

Sealer helps to fill in irregularities and minor discrepancies between the filling and canal walls, accessory canals and multiple foramina. Sealer discloses the presence of ancillary canals, resorptive areas, root fractures, shape of the apical foramen and other structures due to its radio-opacity. A sealer is a good lubricant and helps in the seating of primary cone into the canal. It is a good germicidal or antibacterial.

The ideal requirements for a root canal sealer is

- It should be tacky when mixed to provide good adhesion between it and the canal wall when set, should make a hermetic seal.
- Should have ample setting time, giving the clinician sufficient time to make necessary adjustments to the filling material.
- Particles of powder should be very fine so they can mix easily with the liquid. It should not shrink upon setting.
- All the Sealers shrink slightly upon setting and gutta percha also shrinks when returning from a warmed of plasticized state.
- It was found that zinc oxide eugenol sealers begin shrinking within hours after mixing, but that AH-26 and Endo-fill first expanded and showed no shrinkage for 30 days. The least dimensional change at any time was observed for Endo-fill. Significant dimensional change and continued volume loss can occur in some endodontic sealers.
- Should not stain tooth structure
- Should be bacteriostatic, or at least not encourage bacterial growth
- Grossman tested 11 root canal cements and concluded that they all exerted antimicrobial activity to a varying degree," those containing para-formaldehyde to a greater degree initially. With time, however, this latter activity diminished so that after 7 to 10 days the formaldehyde cements were no more bactericidal than other cements.
- More recently a British group studying the antibacterial activity of four restorative materials reached much the same conclusion regarding zinc oxide eugenol and glass ionomer cement. Another study founded that 10 sealers inhibited growth of streptococcus sanguis and streptococcus mutans.
- Sealers should be insoluble in tissue fluids
- Smith and mccomb found a wide variance in sealer solubility after 7 days in distilled water, ranging from 4% for Kerr's pulp canal sealer to much less than 1% for Diaket.
- Sealer should set slowly
- It should be soluble in a common solvent if it is necessary to remove the root canal filling
- It should not provoke an immune response in periradicular tissue
- It should be neither mutagenic nor carcinogenic.
- It should be tissue tolerant that is non-irritating to periradicular tissue.
- Para formaldehyde containing sealers appear to be the most toxic and irritation to tissue. A case in point is reported from Israel necrosis of the soft tissue and sequestration of crestal alveolar bone from the leakage of para formaldehyde paste from a gingival level perforation

Despite the undisputable improvements, until today no material fulfills all requirements and desirable properties to hermetically seal the root canal system. Apical leakage is still a frequent event in root-filled teeth, which raises concern regarding the quality of obturation provided by the currently available filling materials. There are several methods for evaluating the apical sealing of root canal sealers, such as bacterial penetration, fluid transport, clarification, penetration of radioisotopes, electrochemical methods and gas chromatography. Dye penetration tests, however, seem to be the most widely used.

The purpose of this in vitro study was to compare, using a dye penetration test, the sealing ability of root canal sealers (Zinc oxide eugenol sealer) and three recently introduced sealers (Endosequence, RoekoSeal and Glass ionomer sealer).

Fig 1: Armamentarium required.

Fig 2: Stereomicroscope.

Fig 3: Different types of Sealer used.

Fig 4: Material used for clearing teeth.

Fig 5: Obturation of sectioned teeth using four different sealers

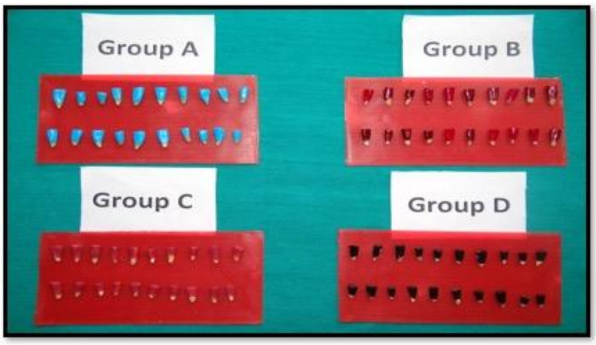

Fig 6: Nail paint application after obturation.

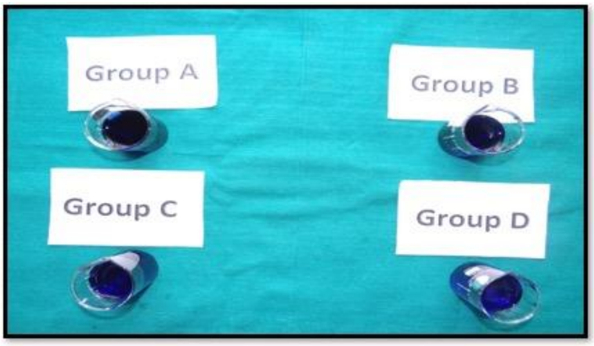

Fig 7: Teeth immersed in methylene blue ink.

Fig 8: Teeth immersed in Nitric acid.

Fig 9: Cleared sample.

Fig 10: Dye penetration under Sterio microscope.

Table 1. Distribution of Samples

Groups	No. Of Samples	Type Of Sealer
Group A	20	Endosequence BC Sealer (Brasseler USA, Savannah, Georgia)
Group B	20	Roekoseal(Roeko,Germany)
Group C	20	Glass Ionomer Cement(GC,Fuji)
Group D	20	Zinc Oxide Eugenol

Table 2: Measurement of dye penetration in Millimetres

S.NO	*ENDOSEQUENCE*	*ROEKOSEAL*	*GC FUJICEM*	*ZINC OXIDE EUGENOL*
1	2.9	0.72	4.27	4.5
2	3.52	0.77	4.1	4.75
3	2.5	1.42	4.22	3.4
4	1.45	3.22	2.1	4.12
5	3.47	1.95	3.55	2.32
6	3.02	2.27	3.55	2.4
7	2.1	1.9	4.27	2.02
8	0.05	1.42	4.8	2.67
9	3.4	0.52	4.7	2.53
10	4.07	1.12	4.5	2.4
11	3.8	0.67	3.12	3.27
12	4.27	2.6	0.17	3.3
13	4.7	3.07	2.27	3.55
14	3.05	2.07	3.55	2.4
15	0.12	1.02	3.47	2.66
16	0.05	0.0	1.87	2.57
17	0.35	1.25	3.62	2.9

Table 3: Mean, Standard Deviation, Maximum and Minimum Values of microleakage (in mm) in four experimental groups.

GROUP	*N*	*MEAN*	*SD*	*MINIMUM*	*MAXIMUM*
Endosequence	20	2.29	1.54	0.05	4.70
Roekoseal	20	1.33	0.97	0.02	3.22
GC Fujicem	20	3.16	1.29	0.17	4.80
Zinc Oxide Eugenol	20	3.17	0.80	2.02	4.75

Table 4: Comparison of microleakage among four experimental groups using Kruskal Wallis ANOVA.

KRUSKAL WALLIS TEST VALUE	*DF*	*P VALUE*
24.089	3	0.000 (<0.001) Significant Difference

Table 5: Comparison of groups (pair wise comparison) for microleakage using Mann-Whitney test.

COMPARISON GROUPS	*MANN-WHITNEY U TEST VALUE*	*P VALUE*
Bioceramic sealer V/S Resin Based Sealer	124.000	0.040 (<0.05) Significant Difference
Bioceramic sealer V/S GC FujiCEM	125.500	0.044(<0.05) Significant Difference
Bioceramic sealer V/S Zinc oxide eugenol	143.000	0.127 (>0.05) Not Significant
Resin Based SealerV/S GC FujiCEM	52.500	0.000 (<0.001) Significant Difference
Resin Based SealerV/S Zinc oxide eugenol	29.000	0.000 (<0.001) Significant Difference
GC FujiCEM V/S Zinc oxide eugenol	191.000	0.808(>0.05) Not Significant

AIMS AND OBJECTIVE

To compare and evaluate the ex-vivo sealing capacity of bio ceramics, resin based and glass ionomer root canal sealers and the amount of apical microleakage among different endo-sealers.

MATERIALS AND METHODS

Sixty single rooted humans freshly extracted teeth to be collected from the Dept. of Oral and Maxillofacial surgery. ***Inclusion Criteria*** Sixty single rooted human permanent teeth, extracted due to periodontal diseases or orthodontic treatment with matured, closed apices with single canal will be included in the study ***Exclusion Criteria*** Teeth with more than one canal, immature root apices, caries involving the root, fracture involving root, thin curved roots, resorbed root.

SAMPLE PROCESSING

Eighty extracted single rooted human permanent teeth are to be randomly divided into four groups of 20 each

Group I - Bioceramic sealer

Group II – Resin based

Group III - Glass ionomer root canal sealer

Group IV- Zinc oxide eugenol

ARMAMENTARIUM USED: Airotor handpiece, Straight micro motor handpiece, Contra-angle micro motor handpiece, Wheel diamond disc, K-files (sizes 15-80) , Endo-motor (Dentsply X-smart)(Fig 1), Protaper rotary files, Stereomicroscope (Fig 2) MATERIALS USED: Glass Ionomer Cement (fuji II) , Normal saline, 1% Sodium hypochlorite, 17% EDTA (Dent Wash, Prime Dental products) , 6% Gutta Percha points, Absorbent paper points, Bioceramic sealer, Resin based, Glass ionomer root canal sealer , Zinc Oxide Eugenol sealer (Fig 3), 1% methylene blue dye (Fig 4), Human single rooted teeth. (Table 1)

MATERIALS AND METHODS

• The extracted teeth were kept in 0.1% aqueous thymol solution under refrigeration.

• Then the teeth were washed under tap water for 24 hours prior to the experiment to eliminate traces of thymol.

• The crowns of all teeth were resected at CEJ, perpendicular to the long axis of the teeth

• Canal length was established by introducing a size 10 K-file into each root canal until the tip of the instrument was visible at the apical foramen.

• Working length was established 1 mm short of the apex.

• The coronal and middle thirds were flared with Gates- Glidden instruments. The canals were prepared using rotary instrumentation (Dentsply Protaper system).

• Irrigation was done with 1 mL of 1% sodium hypochlorite alternated with the same volume of 17% EDTA between instruments.

• After obturation teeth were stored at 37°C, 100% air humidity for 72 hours to allow setting of the sealers.

• Thereafter, three layers of nail varnish were applied to the tooth surface, except for the apical 2 mm, which remained exposed, in such a way that dye could penetrate the canal only via apical region. (Fig 6)

• Each groups were separately immersed in Methylene blue ink (Fig 7) and stored at 37°C for 1 week, after which the teeth are thoroughly washed in running water and the nail polish is to be removed with a scalpel blade.

• Next, the teeth were demineralised using 5% nitric acid (Fig 8) for 72 hours than washed in tap water for 24 hours, dehydrated in ascending concentrations of ethanol at 50%, 70%, 80%, 96% and 100% for 4 hours each, and finally transferred to methyl salicylate for diaphanization.

• The cleared teeth (Fig 9) were analysed by means of a stereomicroscope. The extent of dye penetration is to be measured in millimetres in all aspects of the root canal from the apical stop to the maximum length of dye penetration. (Fig 10)

• Data was analysed statistically using Kruskal-Wallis multiple comparison test. Multiple comparisons were performed using the Mann-Whitney test.

RESULTS

In the present study, eighty extracted human single rooted teeth were collected. They were biomechanically prepared and obturated and divided in four different groups depending upon the sealer used: -

Group A - Endosequence
Group B- RoekoSeal
Group C - GC FujiCEM
Group D- Zinc oxide eugenol

Data were analyzed statistically using Kruskal-Wallis multiple comparison test. Multiple comparisons were performed using the Mann-Whitney test. (Table 2 – 5)

Meaning of results: Kruskal Wallis ANOVA showed significant difference between the groups for microleakage. Hence Mann-Whitney U test was applied for pairwise comparison of microleakege. It showed **significant difference** in microleakege between

Bioceramic sealer and Resin Based Sealer
Bioceramic sealer and Glass ionomer root canal sealer
Resin Based Sealer and Glass ionomer root canal sealer
Resin Based Sealer and Control
Differences were **not significant** between

Bioceramic sealer and Control
Glass ionomer root canal sealer and Control

DISCUSSION

The main goal in endodontic treatment is complete obturation and exclusion of micro-organisms or tissue fluids from the canal system. Once a proper seal is obtained, any inflammatory reaction previously initiated or promoted by noxious matter from within the canal should cease thereafter the periapical lesion should start to resolve.[1]

To achieve this, it is believed that the root canal filling must seal the pulpal space both apically, laterally and coronally to prevent further apical irritation from incomplete elimination of bacterial products and from communication between the apical tissues and the oral cavity. An inadequate seal at the apex is said to account for 60% of failures of root canal therapy.[1]

It is well known that microleakage between the root canal filling and root canal walls may adversely affect the results of root-canal treatment.[2] (Mannocci et al., 1999). Ingle & Taintor (1985) reported that the majority of endodontic failures had been caused by the incomplete sealing of the root canal. Thus, it is necessary to use materials which will be able to create a hermetic seal between the root canal system and the periapical tissues.[3] Therefore, complete obturation of the root canal with an inert filling material and creation of an apical seal have been proposed as goals for successful endodontic treatment (Nguyen, 1984).[2]

Gutta-percha has been shown to have no adhesive qualities to dentine regardless of the obturation technique used (Evans & Simon, 1986). Therefore, sealers are used as a binding agent and as a lubricant to aid in seating the gutta- percha cones and to fill accessory canals (Cohen & Burns, 1991).[4]

Root canal sealers, used alone or in conjunction with solid core filling materials are intended to achieve a fluid tight or hermetic seal. Adhesion to dentinal walls, complete obturation of the root canals apically and laterally and deep penetration to the dentinal tubules is among the main qualities of a sealer.

Per Timpawat et al.[5] endodontic sealers are used to eliminate the interface between the gutta percha and the dentinal walls. An ideal root canal sealer should have low viscosity and good wetting properties to flow into the irregularities on the wall of the root canal and fill the space between the gutta percha cones and surface of the root canal. Leakage may, however occur at the interfaces between the sealer and dentin, sealer and gutta percha and in spaces within the sealer itself. (Cobankara et.al. 2002). Thus, the quality of the filling depends largely on the sealing capacity offered by sealers.[6]

The present study is an in-vitro study based on dye leakage and stereomicroscope. The reason behind selecting an in-vitro study to access and compare microleakage was that most in-vivo studies done on microleakage required animal samples and were time consuming while clinical studies had other limitations that included large number of specimens, feasibility and technique sensitivity. Extracted human teeth were used in the present study to improve the reliability of the investigation by stimulating the clinical situation.[7]

Apical leakage of any substance is prevented by completely filling the root canal with a material

that adheres to dentin, does not shrink and is not water soluble.[8]A sealer combined with gutta percha could be such a material. It might be if more gutta percha and the less 'islands' of sealer which are found, the better would be the result, because of the reduced effect of polymerization shrinkage of the sealer.[8] Gutta percha is currently the obturating material of choice because of its biological and mechanical properties.[9,10]

Root canal sealer was used because it improves the sealing ability of gutta-percha as root filling material, both in clinical practice and experimental research, regardless of the technique used.[7]

Some explanations may be suggested for the lower mean scores of apical leakage in the RoekoSeal group. Firstly, the main content of this material is Polydimethylsiloxane, that is, silicone, which gives the sealer good flowability and high penetrability even in dentinal tubules, as observed in the histologic examination Huumonen et al. (2003). Moreover, RoekoSeal has excellent dimensional stability and is, therefore, superior to materials that contain resin in their composition – like Endosequence BC, GC FujiCEM and Zinc oxide eugenol and that may undergo certain polymerization shrinkage, which results in empty spaces in the obturating mass. The initial expansion observed in the silicone-based sealers may explain the better sealing ability provided by these materials. Finally, the low solubility of RoekoSeal may also have contributed to the minimal leakage scores found in the present study.

The results of the current study are in agreement with the results of a number of ex vivo studies. Wu et al. (2006)[11] found that RoekoSeal was dimensionally stable and prevented leakage for at least 1 year. The authors pointed out that a high gutta-percha/sealer ratio may not be necessary when RoekoSeal is used. In addition, laboratory investigations have reported a 0.2% setting expansion (Orstavik et al., 2001), biocompatibility (Miletic et al., 2005)[11], and acceptable coverage of the root canal wall of RoekoSeal. (Ardila et al., 2003 Additional favourable properties were its fluidity and capacity to set under both humid and dry conditions (Wu et al., 2006).[11] Gencoglu et al. (2003) investigating microleakage in presence of 17% EDTA found the best results in teeth in which RoekoSeal was used. RoekoSeal showed good adaptation to the root canal wall and penetration into dentinal tubules in the middle third of the canal.

Kenneth K et al. (2009)[13] found that setting time of Endosequence BC sealer is 4 hours but can be extended in overly dry canals. The setting time of Endosequence BC sealer is dependent upon the presence of moisture in the dentinal tubules. The pH during the setting process is very alkaline (pH 12.9) which increases its bactericidal properties. Gutta-percha may or may not be required for obturation. Leakage studies constitute a major part of contemporary endodontic research.[14] The use of dye is one of the most common methods for studying microleakage and easily performed.[14]Though the reliability of dye penetration studies have been questioned.[15]the dye leakage method still remains

good test for screening of sealing ability.[16]During last two decades, an increasing number of leakage studies have been published in the endodontic literature. Much emphasis has been placed on 'sealing ability', presumably because it was supposed that the majority of endodontic failures were caused by inadequate root canal obturation.[14]

The linear dye penetration technique does not provide any information about the volumetric data of the tracer penetrating the interface between the root filling and the root canal wall and so therefore to be regarded as a semiquantitative.[15]

One of the major problem with studies of microleakage is the lack of standardization of samples and technique. Therefore, in the present study an attempt was made to make the samples of teeth comparable by using teeth with single root and minimal curvature.[7] This avoided the problem of multiple canals or differences between roots with different curvatures. The teeth were also of similar lengths so that they would have the same volume available for infiltration.[17]Also all the canals were prepared to the same size and a single operator performed the preparation and obturation to try to overcome variation.[7,14]

The basic concept behind the dye penetration technique is that it uses passive diffusion and capillary action in order to penetrate through any spaces between the canal walls and filling material.

Bacteria, dye (eosin, methylene blue, black India ink, Procion brilliant blue and others), radioisotopes and other substances have been employed as tracers for leakage evaluation. Small molecules (for example, water) may leak 100-fold more than large ones(albumin).[18]Although pathogenic substances such as bacteria and their products have a large particle size, molecules of some nutrients for micro-organisms, e.g., sugar are small. If nutrients from the oral environment can enter a filled canal, the bacteria that may persist after thorough cleaning, shaping and obturation may multiply. Thus, the possibility of bacteria and/or their toxic products leaking beyond the apical foramen may be increased. It seems logical that the less leakage the better. Therefore, it appears that root canal fillings that are impervious to small molecules are optimal, and the use of tracers of small molecular size is indicated.

According to various studies conducted methylene blue has been proved to be a useful aid in endodontic therapy. Methylene blue dye was chosen for this study because it exhibits high sensitivity and its particles use of similar size to microorganisms and their metabolites. Methylene blue is inexpensive, easy to manipulate exhibits high degree of staining and molecular weight even lower than that of bacterial toxins. Methylene passes along air-filled gaps by capillary action whereas in water-filled gaps, it passes by diffusion.[19] This method allows to produce sections showing leakage in contrasting colours to both tooth and restoration without the need for further chemical reaction.

Alberg et al [20] have reported that methylene blue is a superior tracer of micro spaces than India ink or Procion. Maltoff et al [21] in a study have demonstrated that methylene blue is more sensitive indicator of leakage that radio isotopes. He also demonstrated that methylene blue penetrated a greater distance along root canal fillings than radio isotopes. Hence in the present study aqueous methylene blue was employed as the tracer. According to Kennedy et al [22] this dye is visible under direct light, penetrates dental structures well and does not react with tissues thus allowing the measurement with a millimetre ruler directly on teeth as recommended by Madison and Krell.[23]

Vies et al [24] have reported that dye penetration dye measurements produce almost the same result as clearing techniques. Clearing technique is currently more popular. However, it has been postulated that prolonged immersion of the specimens in nitric acid and alcohol may cause washing out of tracer [20] Therefore, time duration for immersion of samples in nitric acid and alcohol should be accurate for better results.

In our study RoekoSeal performed better as compare to other sealers as the mean value of RoekoSeal (1.33) was lower than Endosequence (2.29), GC FujiCEM (3.16) and Zinc oxide eugenol (3.17).

CONCLUSION

Following conclusions were drawn from this study:

1. The statistical analysis shows that all materials showed microleakage but there is significantly less microleakage in RoekoSeal (1.33 mm) when compared to Endosequence (2.29 mm), Glass ionomer cement (3.16 mm) and Zinc oxide eugenol (3.17 mm).
2. Microleakage in Glass ionomer cement and Zinc oxide eugenol is more when compared to Endosequence.
3. The above discussion based on this study concludes that RoekoSeal is the better root canal sealer to prevent microleakage.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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