Bacterial Penetration along Different Root Canal Fillings in the Presence or Absence of Smear Layer in Primary Teeth

Sisodia R* / Ravi KS** / Shashikiran ND*** / Singla S**** / Kulkarni V *****

Aims: *To study the effect of the smear layer on the penetration of bacteria along different root canal fillings and to compare the sealing ability of new endodontic material Apexit plus as compared to Zinc Oxide Eugenol (ZOE) in primary teeth.* ***Study design:*** *A total of 60 human root segments were instrumented for endodontic treatment. Half of the sample size was irrigated with normal saline and in other half, 3% NaOCl, 3% H_2O_2 and 17% EDTA was used alternatively as irrigant during instrumentation. The roots were rinsed thoroughly with distilled water and sterilized by autoclaving for 20 min at 121 ± 2 ^{0}C. Roots with and without smear layer were obturated with Apexit plus, Zinc oxide eugenol. Following storage in humid conditions at 37^{0}C for 2 days, the specimens were mounted into a bacterial leakage test model for 180 days.* ***Results:*** *At 180 days, there is statistically significant difference with a P value of < 0.05 among all groups except ZOE –smear and –nonsmear. In the presence of smear layer, Apexit plus demonstrated more leakage. No leakage was observed in ZOE groups. ZOE demonstrated better sealing ability than Apexit plus.* ***Conclusions:*** *Removal of smear layer helps in better resistance to bacterial penetration along Apexit plus root canal fillings but no effect is seen along ZOE root canal fillings.*

Keywords: *Apexit plus, bacterial penetration, smear layer, Zinc oxide eugenol.*

INTRODUCTION

Microbes are considered as the primary etiologic agents in endodontic diseases. The ways of reducing these agents are: root canal debridement, antimicrobial intracanal medicament and antibacterial filling materials. But the complexity of root canal system presents a problem for chemo mechanical preparation.[1] The other factor determining the success of endodontic treatment is the sealing ability of the material and its potent bactericidal action that can maintain tight seal, chemical as well as mechanical along the root canal system.[2] A tight seal prevents entry of microorganism and their by-products to the periradicular area and entomb the remaining microorganism. Hence, helps in healing of apical periodontitis.[3]

A smear layer that is formed on the surface of dentinal walls when the root canals are instrumented.[4] And its significance in endodontics has been the subject of extensive debate since it was first described. It is now generally advocated that the smear layer should be removed prior to the insertion of the root filling.[2] This is assumed to facilitate the adaptation of the filling material to the dentinal walls to improve adhesion and resistance to bacterial penetration. However, the results on smear layer are conflicting and it is unclear whether possible beneficial effects of smear layer removal is a general phenomenon or is dependent on the material and technique used.[5,6] It is therefore of interest to examine whether the adhesive properties as influenced by smear layer, will affect bacterial penetration along different root canal fillings.

In Pediatric endodontics, Zinc oxide eugenol (ZOE) is one of the oldest filling materials for primary teeth since 1953. It has been proved that Zinc oxide eugenol has good sealing ability and bactericidal action[7] but it has a slow rate of resorption and has a tendency to be retained even after tooth exfoliation.[8] Apexit Plus is a new radiopaque, non-shrinking, calcium hydroxide based root canal sealer paste, having slight setting expansion, in combination with the product's low solubility, enables good and durable sealing of the root canal.[9]

The aim of the present study was to evaluate whether the removal of the smear layer aids in preventing bacterial penetration along different root canal fillings and to compare the sealing ability of new endodontic material Apexit plus as compared to Zinc Oxide Eugenol as obturating material in primary teeth.

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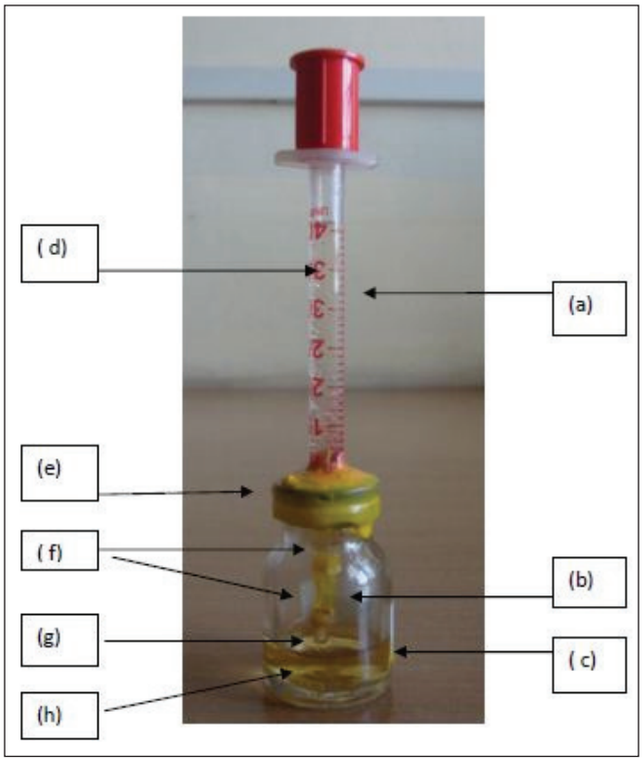

Figure 1. Bacterial penetration model (a) Upper chamber (Insulin syringe) (b) Intermediate chamber (IV fluid tube) (c) Lower chamber (Penicillin glass vial) (d) *Enterococcus Faecalis* bacteria (ATCC 29212 strain) grown in Todd Hewitt broth (e) External seal (CPVC solvent cement) (f) Internal seal (g) obturated root specimen (h) Sterile Brain heart infusion broth (2ml).

Figure 2. Bacterial leakage Model (a) Without and (b) With Turbidity

MATERIALS AND METHOD

A total of 60 single-rooted human primary anterior teeth with 2/3rd root length were stored in 10% formalin after extraction. Deciduous teeth with internal or external resorption leading to perforation were excluded. The crowns were removed and root segments with a standardized length of 7 mm from the cemento-enamel junction were prepared by cutting off the root tips, using a rotating diamond disc under water cooling. The roots were randomly divided into 2 groups based on irrigants to be used into Group I (Smear group) and Group II (Non-smear group). Stainless steel K- file were used to prepare each root canal with selective filing technique to avoid perforation on the resorbed surface of the roots. Normal saline was used as irrigants in group I during shaping and cleaning. In group II, 3% NaOCl, 3% H_2O_2 and 17% EDTA was used alternatively as irrigant during instrumentation, followed by 3 ml rinse with 17% EDTA for 1min in each canal. Finally the roots from both groups were rinsed thoroughly with distilled water and sterilized by autoclaving for 20 min at 121 ± 2° C. Groups I and II were further divided into subgroups based on obturating material i.e. Apexit plus(Ivoclar-Vivadent) and Zinc oxide eugenol (Prime dental products Pvt Ltd.). Zinc oxide eugenol was obturated with the help of reamer and Apexit plus was obturated into canal with the syringe technique. Radio Visio Graphy was taken to ensure the proper obturation of the canal. All obturated specimens were kept in incubator for 48 hrs and were mounted on bacterial penetration model.

Bacterial leakage test

The Bacterial leakage test was done by three chamber modified model described by Saleh *et al*.[10] (Fig. 1). Upper chamber was prepared from insulin plastic syringe, whose needle was detached and piston was removed. Intermediate chamber was consisted of polyethylene based IV fluid tube, length 7mm, to accommodate coronal end of root specimen. The free end of intermediate chamber was connected to upper chamber at the hub portion of insulin syringe, serving as bacterial reservoir. The internal attachments sites were sealed with Chlorinated polyvinyl chloride (CPVC) solvent cement. Lower Chamber was made of sterilized Penicillin glass vial, containing 2ml of Brain Heart Infusion broth. The apical tip of the mounted root specimen from intermediate chamber was hanging vertically 1-2mm into the sterilized brain heart infusion broth, present in the lower chamber. The external attachment site of upper and lower chamber was united with sealing agent. Each model was labelled for its sample number, date, obturating material and irrigant used. An overnight culture of *Enterococcus faecalis* bacteria (ATCC 29212, vancomycin sensitive strain), grown in Todd Hewitt Broth, was added to each top chamber, covered with closing lid. The mounts were kept at 37 ± 1° C throughout the experiment (180 days). The bacteria and medium in the upper chamber were replaced with freshly grown cultures twice weekly to maintain viability and numbers of bacteria. The bottom chambers of all mounts were checked daily for turbidity as evidence for bacterial penetration along the root filling (Fig.2). On observation of turbidity in the lower chamber, the seal was broken, and the nature and purity of the organism growing there were confirmed by cultural morphology on Mueller Hinton agar. The catalase and antibiotic sensitivity test were performed, followed by gram staining and by specific growth

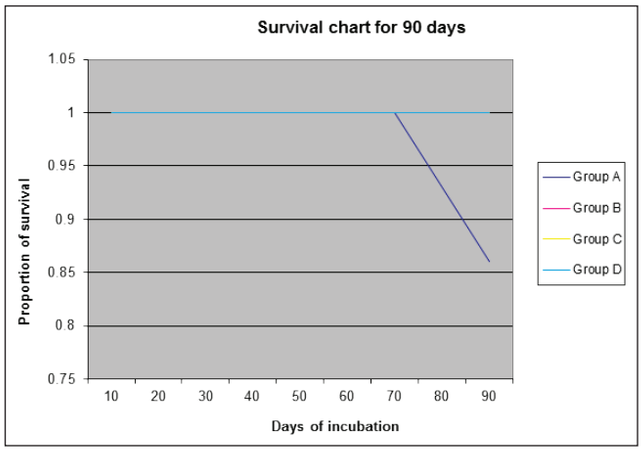

Figure 3. Kaplan Meier plot of leakage showing the proportion of roots resisting leakage in each experimental group over 90 days of time.

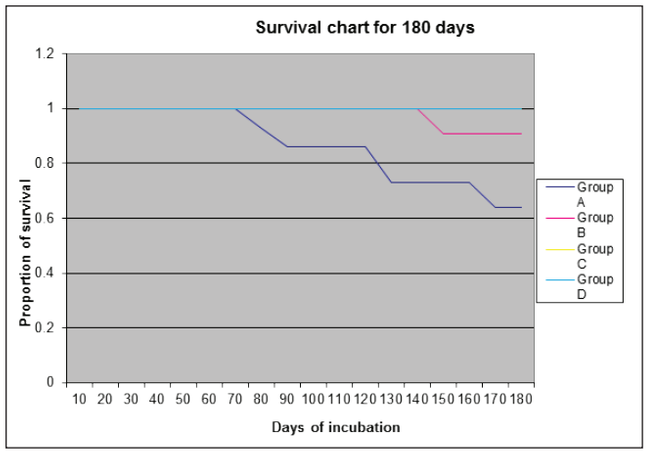

Figure 4. Kaplan Meier plot of leakage showing the proportion of roots resisting leakage in each experimental group over 180 days of time.

on bile esculin agar (fig.3). The day of leakage was recorded for each leaking sample and the number of leaking samples was recorded per group at 90 days and 180 days interval. Statistical analysis was performed using the Kaplan Meier test for survival analysis, which includes calculation of the median time of leakage, and pairwise comparisons of groups with the log-rank (Mantel Cox) test (Table 2 and 3). The P-value of <0.05 was considered to be significant.

RESULTS

The present study consisted of sample size of 60 root specimens; contributing 15 specimens in each four group were tabulated in table 1. The exclusion of few mounted root specimens, not showing true bacterial leakage, due to various reasons during experiment period were tabulated in Table 4 and 5 at 90 days and in between 90 and 180 days respectively. The result of bacterial penetration test of 90 days is summarized in table 2 and fig.3. At 90 days, only apexit plus demonstrated true bacterial leakage in the presence of smear layer but no leakage in the absence of smear layer. No true bacterial leakage was observed in Zinc oxide eugenol group. The result of entire 180 days is summarized in table 3 and fig. 4. Even at the end of the experiment of 180 days, it was observed that there was no true bacterial leakage in Zinc oxide group, irrespective of presence or absence of smear layer. But Apexit plus demonstrated more true bacterial penetration along the root canal in the presence of smear layer than in the absence of smear layer. Hence Zinc oxide eugenol demonstrated better fluid impervious seal than Apexit plus group. At 90 and 180 days, there was statistically significant difference with a P value of < 0.05 among all groups but statistically insignificant difference was observed, with a P value >0.05 between ZOE- smear and ZOE- nonsmear groups.

Table 1. Study groups

Group	material	No. of specimens	code
A	Apexit plus-smear	15	AP-s
B	Apexit plus-nonsmear	15	AP-ns
C	Zinc oxide eugenol -smear	15	Ap-s
D	Zinc oxide eugenol - nonsmear	15	Ap-ns

Table 2. Experimental design and results of leakage at 90 days

	At 90 days		
Group	No. of specimens	Proportion of leaking specimens	Median time of leakage in days
A (AP-s)	14	2/14	90
B (AP-ns)	15	0/15	90
C (ZOE-s)	13	0/13	90
D (ZOE-ns)	12	0/12	90

Table 3. Experimental design and results of leakage at 180 days

	At 180 days		
Group	No. of specimens	Proportion of leaking specimens	Median time of leakage in days
A (AP-s)	11	4/11	180
B (AP-ns)	11	1/11	180
C (ZOE-s)	10	0/10	180
D (ZOE-ns)	10	0/10	180

DISCUSSION

There is a lack of literature for evaluating the effect of smear layer in resisting bacterial leakage in primary teeth with different root canal fillings. Hence, the present study was carried out to evaluate the fluid impervious seal by the mean of bacterial penetration test.

Smear and nonsmear group were divided based on the irrigants to be used during instrumentation. Under clinical conditions, especially during the treatment of infected teeth, viable bacteria and their products can be incorporated into the smear layer, forming a deposit of irritants.[11] Therefore, its complete elimination would allow the most effective removal of irritants from root canals, besides promoting an increase in the dentin permeability and the contact surface between the dentin and the filling paste. This contributes greatly to the success of the endodontic therapy.

Table 4. Exclusion of specimens during 90 days experimental time.

Groups	No. of specimens excluded due to different reason at 90 days		
	Contamination from external source	Technical failure or internal seal failure in model	Very early result due to human technical problem
A	0	0	1
B	0	0	0
C	0	1	1
D	1	0	2

Table 5. Exclusion of specimens during 90 to 180 days experimental time.

Groups	No. of specimens excluded due to different reason at 180 days	
	Contamination from external source	Technical failure or internal seal failure in model
A	1	0
B	3	1
C	2	1
D	2	0

To obtain the complete removal of the smear layer, that is, both organic and inorganic components, the combined use of NaOCl and EDTA is recommended.[12] The chelating agent prepares the canal wall surfaces so that cleansers and medications are effective with their antibacterial action.[13] Teixiera *et al*.[14] demonstrated that canal irrigation with EDTA and NaOCl for 1, 3 and 5 min were equally effective in removing the smear layer from the canal walls of straight roots. Different irrigation regimens have been proposed to enhance the effectiveness of NaOCl in disinfecting the root canal system. Grossman[15] suggested the alternate use of NaOCl and hydrogen peroxide for the irrigation of the root canal. De Quieroz *et al* [16] found that the combination of sodium hypochlorite and hydrogen peroxide can be used as an alternative disinfectant and/or biofilm remover of contaminated food processing equipment.

Erdemir *et al* [17] and Yiu *et al* [18] found that NaOCl is a strong oxidizing agent and may cause problems when used as the last irrigant. It leaves behind an oxygen-rich layer on the dentine surface, which results in reduced bond strengths and increased microleakage. Therefore, Lai *et al* [19] has proposed to use NaOCl first, followed by EDTA for removal of the smear layer after the instrumentation, and then distilled water as a final rinse in order to minimize the compromising effect of NaOCl. Also, there is better adhesion of the sealer by permitting penetration of sealer into dentine tubules as proposed by Eldeniz *et al*,[20] a procedure which was followed in the present study.

Obturating materials used in this study were Zinc Oxide Eugenol and Apexit plus. Zinc Oxide Eugenol [21] is the most commonly and easily available obturating material for primary teeth. Apexit plus is chosen as this is the calcium hydroxide based paste having resorbable property of excess material beyond the apex, also it is insoluble in water or fluid condition. Apexit Plus is an improved version of Apexit, which has been successfully used in clinical situations since 1990. The main difference in the two formulations is the heightened hydrophilic property of the new product. So, this is the first time in literature where Apexit Plus was used in primary teeth to evaluate fluid impervious seal and the effect of smear layer on adhesion, and the microleakage of Apexit plus.

Bacterial penetration model used in this study is based on model, as described by Saleh *et al* (2008). It was slightly modified as Saleh *et al* [10] used sticky wax as sealing agent for the model and in present study, CPVC solvent cement was used as sealing agent. CPVC Solvent Cement, available in tube forms, is medium bodied, fast setting, high strength for all classes and schedules of PVC pipes and fittings. It was easy and accurate to apply for sealing the model as compared to sticky wax on smaller surface area of primary anterior teeth.

E. faecalis was chosen as the test bacteria, as they are part of normal flora in humans and are frequently isolated in failed endodontically treated primary teeth together with other facultative anaerobes.[22]

Kaplan Meier survival analysis demonstrated highest proportional survival limit of 1 in Zinc Oxide Eugenol group, with and without smear layer throughout the experiment. This may be due to Zinc Oxide Eugenol sets as a hard mass and does not penetrate the dentinal tubules. While Apexit plus- smear group demonstrated 0.64 proportional survival limits at the end of the experiment and Apexit plus- nonsmear group demonstrated 0.91 proportional survival limits at the end of experiment. In the absence of smear layer, Apexit plus demonstrated better resistance to bacterial leakage than in the presence of smear layer. The result of the present study was in accordance with Kokkas *et al* [23] who studied the **effect** of the **smear layer** on the penetration depth of three different **root canal** sealers into the dentinal tubules. Examination under scanning electron microscope revealed that the **smear layer** obstructed all the sealers AH plus, Apexit, and Roth 811 from penetrating dentinal tubules and thus more bacterial leakage. In contrast, **smear layer** removal allowed the penetration of all sealers to occur to a varying depth. These findings suggest that **smear layer** plays an important role in sealer penetration into the dentinal tubules, as well as in the potential clinical implications.

In the present study, Zinc Oxide Eugenol when used as obturating material in primary teeth in the presence or absence of smear layer, showed no bacterial leakage. There was no statistical significant difference between the two groups. The results of the present study

are supported by Tennure *et al* [24] who observed Zinc Oxide Eugenol pulpectomy with smear layer removal in primary incisors exhibited, after 36 months a high success rate; however, comparable results were obtained when the smear layer was not removed. The results are not in accordance with studies done by White *et al* [25] and Kouvas *et al*,[26] who proved that smear layer, affects the bacterial leakage. On the other hand, Apexit plus - smear group showed significant more bacterial leakage than Apexit plus - nonsmear group. The result of the present study is in contrast with the study done by Saleh *et al* [10] who concluded that removal of the smear layer did not impair bacterial penetration along root canal fillings.

In present study, ZOE showed better resistance to bacterial leakage than Apexit plus. This may be due to inherited voids in Apexit plus. Other factors, such as antibacterial properties and physical hindrance, may operate in resisting bacterial leakage. Mutal *et al* [27] concluded that pores and vacuoles were a consistent finding in set sealers. Their frequency and size depended on the density of the sealer and the voids increased when the sealers contained calcium hydroxide. So, this may be the reason for more bacterial penetration in Apexit plus group. ZOE is one of the most widely used preparations for primary tooth pulpectomies. Clinical studies conducted on animals and humans have shown the success rate of ZOE paste used alone to range from 65-95%.[28]

The results of the present study support the view that removing the smear layer in Apexit plus root canal filling is beneficial in preventing bacterial leakage. But retaining or removing the smear layer from the root canal walls has no effect on ZOE root canal fillings. Hence, effect of smear layer removal also depends on the type of material used. Zinc Oxide Eugenol is easily available, cost effective, better antimicrobial property, good plasticity and insoluble to water but it has a slow rate of resorption[8] and has a tendency to be retained even after tooth exfoliation.[29] In some cases unresorbed material has been found to cause deflection of the succedaneous tooth. Calcium hydroxide, despite its antiseptic and osteoinductive properties,[30] has a tendency to get depleted from the canals earlier than the physiologic resorption of the roots.[31] So, both ZOE and calcium hydroxide based root canal filling material have their own advantages and disadvantages. Long-term evaluation, however, need to be further studied.

CONCLUSION

Within the limitation of bacterial penetration model, fluid impervious seal in primary root canal fillings in the presence or absence of smear layer has been evaluated. ZOE has shown better fluid impervious seal than Apexit plus. Removal of smear layer during shaping and cleaning helps in better resistance to bacterial penetration along Apexit plus root canal fillings but no beneficial effect of retaining or removing the smear layer from root canal walls is seen along ZOE root canal fillings.

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