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Evaluation of the Setting Time, Sealing Ability and Flow of Zinc Oxide Eugenol Based and Epoxy Resin Based Endodontic Sealers with and without the Addition of Triple Antibiotic Powder.

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ABSTRACT

The present study evaluated the effect of the determined proportion of triple antibiotic powder(TAP) on the setting time, sealing ability and flow of a zinc oxide eugenol based sealer (ZOE) and a resin based sealer. The proportion of TAP to be added to sealers for maximum antibacterial efficacy against Enterococcus faecalis was determined using agar disc diffusion method. The most clinically effective and usable proportion (90% sealer and 10% TAP) was selected to evaluate the setting time, flow and sealing ability. The results of the setting time, flow and sealing ability were statistically analyzed using ANOVA and post hoc Bonferroni test. ZOE sealer +10% TAP showed decreased setting time and increased sealability when compared toZOE sealer, but the difference was not statistically significant. Nonetheless, the addition of TAP to ZOE sealer significantly decreased the flow. Resin based sealer +10% TAP showed statistically significant decrease in sealability as well as significant decrease in the flow properties when compared to resin based sealer.ZOE sealer with 10% TAP had better antibacterial efficacy with acceptable clinical properties. However, the combination of epoxy resin based sealer and 10% TAP should be used with caution.

Keywords: root canal sealer, triple antibiotic powder, setting time, flow, apical leakage.

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INTRODUCTION

Root canal infections are polymicrobial in nature and the infected root canal system provides a unique niche for the selective species of microorganisms.[1] The success of endodontic treatment is directly related to the decrease in the number of root canal microorganisms.[2]Mechanical instrumentation alone does not result in a bacteria-free root canal system because of the complex anatomy of the root canal system.[3] Therefore, some form of irrigation and disinfection is necessary to remove microorganisms.[4] After chemo mechanical preparation, it's important for the root canal filling material to possess antimicrobial activity to eliminate the remaining microbes and to prevent new bacterial growth.[5]

Root canal sealers have been used along with solid core obturating materials to enhance the fluid-tight seal.[6]Additionally, sealers having antimicrobial properties will play an important role in endodontic success. [7] Zinc oxide eugenol based sealers have been traditionally the most commonly employed sealers. They have served as the benchmark with which other sealers are compared, as they reasonably meet most of Grossman's requirements for sealers.[8] Epoxy resin-based sealers were introduced because of its advantages, such as high radiopacity, low solubility, slight shrinkage and antimicrobial efficacy.[9] Among the resin-based sealers, AH Plus sealer (DentsplyDeTrey, Konstanz, Germany) has shown favorable properties and is widely used.[10]

The complexity of the root canal infection necessitates a combination of antibiotics to address the diverse flora encountered in root canal infections, also decrease the likelihood of the development of resistant bacterial strains.[11] Triple antibiotic paste (TAP) is a mixture of metronidazole, ciprofloxacin and minocycline.[12,13] Triple antibiotic paste (TAP) has been found to have antimicrobial properties and is shownto be biocompatible.[14] It is demonstrated that this mixture is useful for the sterilization of infected root dentine, and that the drug mixture can be applied to root canals.[12] Most endodontic failures are attributed to inadequate debridement of the root canal and the egress of bacteria and other antigens into periradiculartissues. Therefore, root canal sealer in addition to having good sealing ability and biocompatibility also should "ideally" have antibacterial activity to prevent bacterial growth.[15]Hence, combining TAP with the commonly used root canal sealers can be considered.The proportion of TAP added to the sealer could have an effect on antimicrobial properties and physical properties of the sealers.

The present study was conducted to determine the proportion of triple antibiotic powder to be added to zinc oxide eugenol (ZOE) based and epoxy resin based sealers for optimum antibacterial efficacy against *Enterococcus faecalis* (*E. faecalis*), a microoragism commonly seen in persistant root canal infection. The objective of the study was also to evaluate the effect of the determined proportion of triple antibiotic powder on the setting time, sealing ability and flow of these sealers.

MATERIALS AND METHODS

Prior to the commencement of the study institutional ethical committee clearance was obtained (Protocol ref no 12031).

Antibacterial action: *E. faecalis*(ATCC29212) was obtained from the American Type Culture Collection. Thebacterium was grown and maintained on Brain Heart Infusion (BHI) broth (Titan Biotech Ltd. Delhi, India). To preserve the bacterium and its characteristics, upon receipt, cultures were frozen (- 20°C) in vials with glycerol from which new stock cultures were periodically established. A culture of *E. faecalis* was grown overnight at 37°C in BHI broth. Bacterial growth was checked by changes in turbidity at 24 hours. Sixteen agar plates were inoculated with *E. faecalis* by spreading the culture over the surface of the plate with a cotton swab to develop a lawn of cells. The ciprofloxacin (Ciplox 500mg tablet, Cipla, India) metronidazole (Flagyl 400mg tablet, Nicholas, India) and minocycline (Minoz, 100mg tablet, Ranbaxy, India.) powder were prepared separately by removing the enteric coatings of the tablet and grinding the contents to a fine powder using mortar and pestle. These three antibiotic powders were weighed separately in a digital weighing machine (Santoriosanalytic ,Cole-Parmer,USA) to obtain a 1:1:1 proportion for mixing together to obtain triple antibiotic powder.

Zinc oxide eugenol (Pulpdent sealer; Pulpdent Corporation ,USA) and epoxy resin (AH plus, Dentsply DeTrey, Konstanz, Germany) sealers were mixed according to manufacture's instruction, then weighed and mixed with 0%, 0.1%, 0.5%, 1%, 10%, 25%, and 50% concentration of triple antibiotic powder. Sterile paper discs, 6 mm in diameter, were saturated with each of the various sealer antibiotic combinations by exposing the paper discs to the sealer mixture until uniform coverage was visually achieved. The saturated paper discs were placed directly onto the agar plates. Each agar plate contained five paper discs of same sealer antibiotic combination. The BHI agar plates containing experimental and control groups were then were incubated at 37°C for 24 hours. The diameter of zones of inhibition was measured using a digital caliper and recorded in millimeters. The data collected was analyzed using Kruskal-Wallis test and post hoc Mann-Whitney test and p value was set at $p<0.05$ with SPSS, version 16.0 (SPSS Inc., Chicago, IL)

90% sealer and 10% TAP had good antibacterial property and clinical usability. Hence the second part of the study was commenced with this combination.

The study design comprised of four groups as shown in **Table 1**. The respective sealers in Group II and IV and TAP were weighed in a digital weighing machine to obtain a combination of 90%sealer and 10% triple antibiotic powder and mixed. These combinationswere evaluated for the setting time, sealing ability and flow.

Table 1: Sealer and sealer and TAP experimental groups

	Experimental groups
Group 1	Zinc oxide eugenol sealer
Group 2	Zinc oxide eugenol sealer +10% triple antibiotic paste
Group 3	Epoxy resin sealer alone
Group 4	Epoxy resin sealer +10% triple antibiotic paste

Setting time: Setting time was determined using an indentation test according to ANSI/ADA Spec.No.57.Ten samples for each group were prepared using stainless steel moldinternal diameter of 10 mm and a thickness of 2 mm. The mixed sealers from the respective study groups and placed in a humidifier at 37^0c and 95% humidityfor about 150 ± 10 s after the start of mixing. The manufacturer's recommended setting time was considered as the standard time. The indentation procedure was carried out until the Gilmore needle failed to indent the sealer surface. The time elapsed between the start of mixing and the time when the needle completely failed to make an indentation in the sealer was considered.

Sealing ability: Forty single-rooted permanent premolar teeth were root canal treated. The roots were enlarged up to size #F3 using Protaper Universal Ni-Ti files (DentsplyMaillefer, Ballaigues, Switzerland) with copious irrigation with 2.5% sodium hypochlorite and with a final rinse of 17% EDTA for smear layer removal. The dried canals were then coated with a root canal sealers as per the study group. The canals were then obturated with a Size #F3 cone using a single cone technique. The access cavity was sealed with temporary restorative material. The teeth were then placed in a humidifier with 100% relative humidity for one week. Later the teeth were dried and two coats of nail varnish was applied all over, except for the apical 2-3millimeter. The apical portions of the teeth were immersed in 2% Rhodamine B dye (LobaChemie P. Ltd., Mumbai, India) solution for a period of 10 days. The teeth were then rinsed and dried. The teeth were then sectioned into two halves by placing longitudinal grooves on to the labial and palatal root surfaces of the teeth using a diamond disc without entering the pulp space. Sectioning was completed by placing the tip of a chisel in the groove and gently separating the teeth into two halves. The gutta-percha was then removedand canal space was examined under stereomicroscope(Zeiss,Thornwood ,USA)at 30x magnification for the highest level of dye penetration. Once the highest end of penetration was identified the image was captured by the camera (Sony Cyber- Shot, Japan) attached to the microscope (**fig 1**). The captured images were evaluated using Image J software (version -1.410/Java1.6.0-10) with a line tool to determine the apical dye penetration in millimeters.

Flow: The flow of the sealers from the experimental groups was determined using two plate test according to ANSI/ADA specification No.57. 0.05ml of experimental sealers were deposited on the center of the 10X10cm clean glass surface and after three minutes from the beginning of spatualtion another glass plate with 100 grams load was applied over the deposited sealer to allow the formation of a circular disc of sealer. Ten minutes later the additional load was removed and three different diameters were measured in three different directions around the formed disc of sealer using a digital caliper and arithmetical average was calculated. Two conditions were necessary to validate the tests: the difference between the largest and smallest diameter should not exceed 1.0mm and the disc should be uniformly circular.

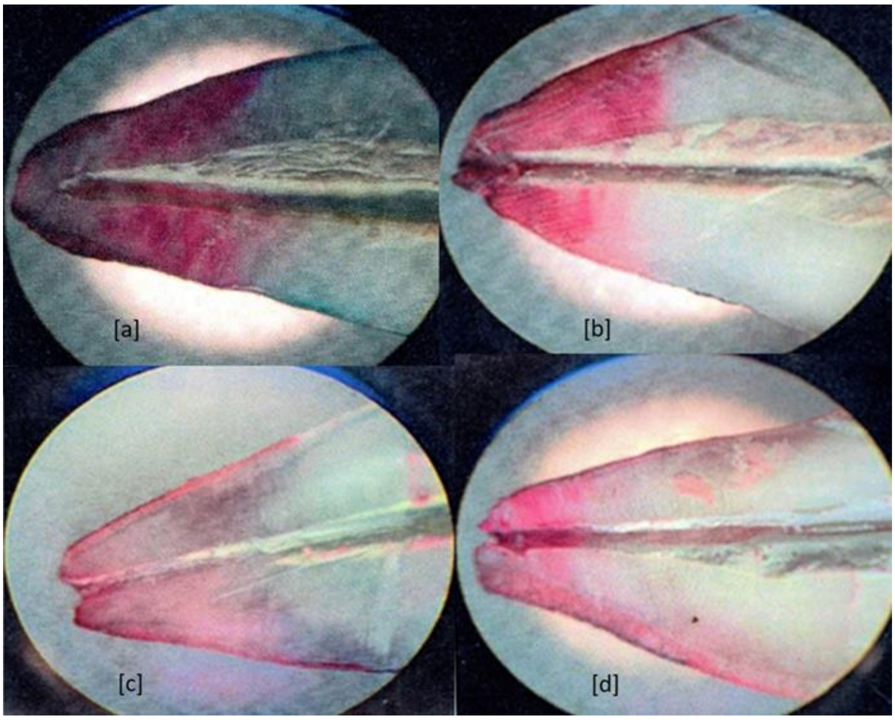

Figure 1: Stereomicroscopic images of dye penetration into the canal space after obturation with guttapercha and various sealers (a) ZOE sealer alone (b) 90% ZOE + 10% TAP (c) AH plus sealer alone (d) 90% AH Plus + 10% TAP.

Statistical analysis: The statistical analysis was carried out using SPSS Version16.0 (SPSS Inc., Chicago IL). The statistical test for setting time, sealing ability and flow was done using ANOVA and post Bonferroni with p value set at <0.05.

RESULTS

Maximum antibiotic sensitivity was shown by 100% triple antibiotic powder (28.4mm) and least by both sterile disc and 100% AH Plus sealer (6mm) (**fig 2**). Among sealer and Triple antibiotic combinations (TAP), maximum antibiotic sensitivity was shown by 50% sealer and 50% TAP. Even though the antibiotic sensitivity shown by 50% sealer +50% TAP and 75% sealer + 25% TAP was more than90%s sealer +10% TAP, the consistency obtained was not clinically suitable. Therefore, 90%sealer +10% TAP combination was used for evaluation of setting time, sealing ability and flow.

Setting time: The setting time of the sealers have been mentioned in **Table 2**. Among the ZOE sealers, Group II (269min) showed lesser setting time compared to Group I (273 min), but the difference was not significant (p>0.05). Whereas, the TAP modified AH plus sealer (Group IV) showed statistically significant increase in the setting time when compared to AH plus alone (Group II) (p <0.001).

Table 2: Descriptive statistics of setting time, sealing ability and flow.

		Setting time			Sealing ability			Flow		
	N	Mean	SD	P value	Mean	SD	P value	Mean	SD	P value
Group1	10	273.10[a]	11.82	<.001	3.23[a,b]	.67	<.003	25.56[a]	1.10	<.001
Group II	10	269.70[a]	8.14		2.56[a]	.58		22.21[b]	1.81	
GroupIII	10	610.50[a]	16.24		2.32[a]	.80		43.26[c]	1.79	
Group IV	10	622.90[a]	11.69		3.50[b]	.89		35.66[d]	2.93	

Groups with the same superscript represents statistically significant difference(p>0.05)

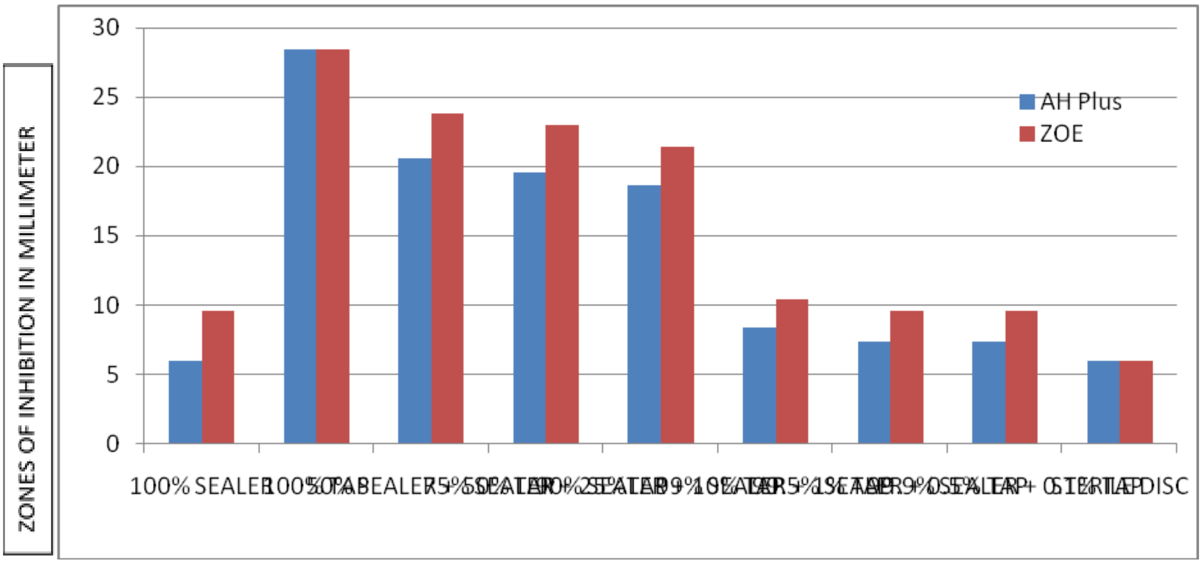

Figure 2: Zones of inhibition of sealer and Triple antibiotic combination

Sealing ability: Group III, i.e. the experimental ZOE sealer showed minimum leakage (2.32 mm), where as Group IV, i.e. the experimental epoxy resin sealer showed maximum leakage (3.50mm).When comparing the sealing ability of these sealers, significant differences were observed in apical dye penetration between Group II and IV ($p<0.05$) and Group III and IV ($p <0.05$) **(Table 2)**

Flow: In this study, the maximum flow was observed in Group III(43.26mm) and least in Group II(22.21mm).Also, there were significant differences seen in flow values between Group III and I , Group II and IV($p <0.05$)**(Table 2)**.

DISCUSSION

In the present study equal amount of metronidazole, ciprofloxacin, and minocycline were mixed together (1:1:1) to make the TAP mixture. This proportion was suggested by Sato, *et al.*[12] and is being widely used. The rationale of using TAP is that, no single antimicrobial agent can be used appropriately for the treatment of mixed infections. Additionally, the infected root canal is inaccessible to the local immune system and the concentration of the drug that reaches the canal space after administration of systemic antibiotics is likely to be minimal and thus insufficient to inhibit the bacterial growth. Therefore, local administration of antibiotics within the root canal system may be a more efficient mode to control the microbial invasion.[10]

In the current study, zinc oxideeugenol base sealer in combination with TAP showed more antibacterial efficacy when compared to epoxy resin based sealer + TAP. The eugenol is a potent antimicrobial agent, and therefore, the activity of ZOE- based sealers may be attributable to the free eugenol released from the set materials.[16]The result of the present study was consistent with that of Gomes *et al.*[17] which showed ZOE-based sealer such as Endofill and Endomethasone demonstrated thehighest antimicrobial activity than epoxy resin-based sealer such as AH-Plus and AH- 26. Conflicting results have been reported by Mohammadi and Yazdizadeh[18]who found that, AH-26 had the largest inhibition zone in comparison with ZOE sealers.The difference in microorganism strains used may be the main reasons of these controversies.[19]Kaiwar, *et al.* [20] found that AH Plus sealer had no zones of inhibition against *E. Faecalis* and they suggested that the elimination of formaldehyde from epoxy resin sealer has made it ineffective against *E. feacalis.*The properties of root canal sealers have an impact on the quality of the final root filling. The laboratory studies on the physiochemical properties could contribute to a better understanding of the clinical behavior and handling performance of the endodontic sealer.

In this study Group II (90%ZOE with 10%TAP) showed less setting time when compared to Group I (ZOE) though not statistically significant(>0.05).Therefore, it shows that addition of TAP does not have much effect on setting time of ZOE sealer. The decrease in setting time might be because of the variation in the powder-liquid ratio as the powder form of TAP is added to ZOE. Similarly, AH Plus sealer with TAP showed higher setting time than AH Plus sealer alone but not significant. Thisindicates that addition of TAP does not have much effect on setting of AH Plus sealer. The increase in setting time might be due to interference in the polymerization reaction of the resin caused by the addition of TAP.

When comparing sealing ability all the groups showed apical leakage and significant difference were seen among groups (Table 2).AH- Plus is an epoxy resin based sealer and though to be able to react with any exposed amino groups in collagen when the epoxide ring opens, thus having higher bonding to dentin.[21] Leakage of AH-Plus sealer may have resulted from inadequate bonding between the sealer and gutta-percha point, allowing fluid to flow at the interface.[22]Whereas ZOE based sealer may have showed leakage due to poor adhesive properties to dentin. [23]These results are consistent with the study by Adanir, *et al*.[24] ,Kumar, *et al*.[25],De Almeida, *et al*[26]and Venturi, *et al*.[27].ZOE +10% TAP showed significantly lesser dye penetration compared to AH Plus +10%TAP (Table 2). The increase in microleakage could be attributed to the interference of TAP during the polymerization reaction of the epoxy resin based sealer.

All the sealer groups showed flow more than the minimum recommended by the ANSI/ADA(20mm).The maximum flow was observed in group III(AH Plus sealer) and group II(ZOE+10%TAP) showed minimum flow. AH Plus +10%TAP showed significantly more flow when compared to ZOE+10%TAP ($p<0.01$) (Table2).Epoxy resin is the component responsible for providing flow to resin based endodontic sealers. However Kaplan, *et al.*[28]assessed the flow of five endodontic sealers and concluded that the factor determining flow may not be its composition, but their final consistency and setting reaction. AH-plus +10%TAP showed less flow when compared to AH Plus and the difference was statistically significant($p<0.01$). The reduction of flow following the addition of TAP to epoxy resin and ZOE resin sealer could be attributed to the physical form size and chemical composition of the antibiotic powder. The antibiotic mixture in powder form had particles of different particle size than the sealer component. Its incorporation would have altered the powder/liquid ratio altering their viscosity. The rationale of this study was to offer the clinician an insight regarding the addition of TAP to sealers. Long-termstudies are needed to evaluate the discoloration potential and the effect on the mechanical properties of dentin.

CONCLUSION

- Combining 10% TAP to ZOE sealer did not significantly affect the setting time and sealing ability. The flow of the experimental ZOE sealer was within the recommendable limit.
- Combination of epoxy resin based sealer with 10% TAP did not affect the setting time and the flow, however it significantly decreased the sealing ability.
- ZOE sealer with 10% TAP had clinically acceptable properties. However, the antibiotic modified epoxy resin based sealer should be used with caution.

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