

A Comparative Evaluation of Sealing Ability, pH and Rheological Properties of Zinc Oxide Eugenol Sealer Combined with Different Antibiotics: An In Vitro Study

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ABSTRACT

Aim: The objective of this study was to evaluate and compare the sealing ability, pH & viscosity of Zinc oxide eugenol sealer mixed with different antibiotics (Amoxicillin, Ciprofloxacin, Clindamycin and Doxycycline).

Materials and Methods: Ninety single rooted anterior teeth were randomly divided into four experimental groups of 15 teeth each and 3 control groups of 10 each. Sealer-antibiotic combination was used as a sealer in experimental group. Teeth were sectioned longitudinally to assess linear dye penetration. pH and viscosity of the experimental groups were evaluated with Elico pH Meter and Brook Field Viscometer respectively.

Results: (Amoxicillin+ ZOE) Group II and (Clindamycin + ZOE) Group IV have shown minimum linear dye leakage of 1.07mm & 1.22 mm respectively & (Ciprofloxacin + ZOE) Group III and (Doxycycline + ZOE) Group V 2.69 mm & 2.59 mm respectively. There was decrease in the viscosity of the experimental group. pH was found to be 8.55 for Group II sealer which was greater than control group.

Conclusion: Amoxicillin and sealer combination can improve the success rate of endodontic therapy by enhancing the antimicrobial activity, alkaline environment and reducing apical microleakage.

Keywords: Apical seal, GP – gutta-percha, Methylene blue, Microleakage, Stereomicroscope, Shear stress, Shear rate, ZOE- zinc oxide eugenol, ZnO- zinc oxide

INTRODUCTION

As the primary aetiological factor is infection, endodontic treatment is directed at control and elimination of the root canal flora by working in a sterile way [1,2]. With all the advances in endodontic techniques, we still lack a perfect system that can render complete eradication of microorganism from the root canal system [3] as the root canal system has many variations in anatomy such as irregularly shaped canals, lateral and accessory canals, a variety of fins which lead to difficulty in preparing the canals to a form conducive to complete obturation with filling materials [4,5]. To prevent bacterial growth, ideally, obturation materials and sealers should have antimicrobial properties [6,7]. Some of the sealers with antibacterial properties are seal apex, apexit, AH plus, apexit plus and tubliseal but have poor sealing ability including calcium hydroxide based sealers [8].

Systemic antibiotics cannot reach the entire infected area due to insufficient blood supply. Intra canal antibiotics may be more efficient and possibly superior to systemic administration as this will avoid side effects and permit higher concentration of antibiotic to reach the infected area. Zinc-oxide-eugenol based sealers have some antibacterial activity of their own; also exhibit some toxicity when placed directly on vital tissues. However, ZOE sealers have long been considered the sealer of choice in endodontic therapy. They get absorbed if extruded out of periradicular tissues and have anti-inflammatory properties [9]. The antibacterial effect ZOE sealers have is not sufficient to totally eradicate the microbiota from the root canal system. Addition of antibiotics to the sealers may be another option to increase the antibacterial effect of the sealer [10]. Amoxicillin, ciprofloxacin, clindamycin and doxycycline were selected because of their broad spectrum activity [11,12]. Combining antibiotics with endodontic sealer have definitely improved the antimicrobial efficacy, but no such emphasis was made on the physical properties and sealing ability of endodontic sealer when mixed with antibiotics [7].

So the question arises whether there is an alteration in the physical properties of ZOE sealer if antibiotics are added. Sealers need alkaline pH for appropriate clinical application in root canals. Flow measurements evaluate the ability of sealer to fill accessory canals, main canals and the space between gutta-percha points [13].

So this study was undertaken to evaluate the sealing ability of root canal sealers when mixed with different antibiotic powder (Amoxicillin, Ciprofloxacin, Clindamycin and Doxycycline) and to evaluate the difference in pH and viscosity of sealer when antibiotics were added to them.

MATERIALS AND METHODS

Ninety non-carious extracted single rooted teeth were selected (Vishnu dental hospital, Bhimavaram, 2013). They were randomly divided into 4 experimental groups of 15 teeth each and 3 control groups of 10 teeth each. Teeth were stored in 10% formalin until use. Crowns were sectioned at the amelo-cemental junction with a water cooled double sided disc and diamond fissure bur at high speed {430k rpm}.

Group I:- Control teeth were obturated with Gp and ZOE sealer.

Group II:- Teeth were obturated with Gp and AMOXICILLIN + ZOE sealer.

Group III:- Teeth were obturated with Gp and CIPROFLOXACIN + ZOE sealer.

Group IV:- Teeth were obturated with Gp and CLINDAMYCIN + ZOE sealer.

Group V:- Teeth were obturated with Gp and DOXYCYCLINE + ZOE sealer.

Group VI:- Positive control teeth were obturated with Gp without sealer.

Group VII:- Negative control teeth- apices were sealed with nail varnish.

The working length was determined by introducing #25 K file until it appeared at the apical foramen. Canals were prepared using step back technique with K&H files(Sybron Endo) along with irrigation with 3% NaOCl and RC Help (Prime Dent Product Ltd. Thane, India). Sealer samples were prepared by adding 10% of wt. of pure antibiotic to zinc oxide powder and hand mixed with mortar and pestle. To this, eugenol was added (powder / liquid ratio - 1.1 gm). Zinc oxide powder to 0.5 ml. eugenol liquid) [14] and mixed to a paste consistency. ZnO/antibiotic ratio was 10:1. The canals were coated with sealer with lentulo spirals and the obturation was done by cold lateral condensation technique. The access cavities were sealed with Coltosol (Coltene, Whaledent). Radiographs were taken using radiovisiography (RVG) for all the teeth in both mesiodistal and labiolingual directions to evaluate the technical quality of root canal obturation. The roots were placed in physiological saline at 37°C for 48 h to ensure that the sealer sets in an environment similar to that of invivo.

The roots of all teeth were dried and coated with two layers of nail varnish except for apical 3mm except negative control teeth, where entire root surface was varnished. Teeth were transferred to vials of 2% methylene blue solution and stored till 7 d. After removal from dye, teeth were sectioned longitudinally, labiolingually and apical microleakage was assessed by measuring linear dye penetration using stereomicroscope (Olympus S Z X16).

Estimation of pH of ZOE and antibiotic combination was done according to formulation- 1gm of ZnO powder and 0.5 ml of eugenol [14]. Sealer samples were prepared by adding 10gms of zinc oxide powder to 5ml of eugenol and were freshly mixed which was minimum required quantity for immersion of pH electrode of pH meter (ELICO pH METER L1 120 DIGITAL). For Experimental group, 10% of wt. of antibiotic powder was added to ZOE and pH was evaluated. BROOK FIELD viscometer (rotational viscometer) was used to evaluate the rheological properties. 100ml of ZOE mix was required for immersion and rotation of spindle of viscometer. Hence, 120gms of zinc oxide, 60ml of eugenol and 10% of antibiotic powder were taken to evaluate the viscosity of sealer with antibiotic combination.

To assess the sealing ability of the ZOE and antibiotic combination which was observed under stereomicroscope, statistical analysis performed are one-way ANOVA test, Post-Hoc-test Tukey HSD and Independent sample t-test.

RESULTS

The results of sealing ability seen under stereomicroscope 2.5X are as shown in [Table/Fig-1].

Source of variation	Sum of squares	Degrees of freedom	Mean sum of squares	F- ratio	p- value
Between groups	24.04	2	12.0214	8.4558	0.0008 S
Within groups	59.71	42	1.4217	****	****
Total	83.75	44			

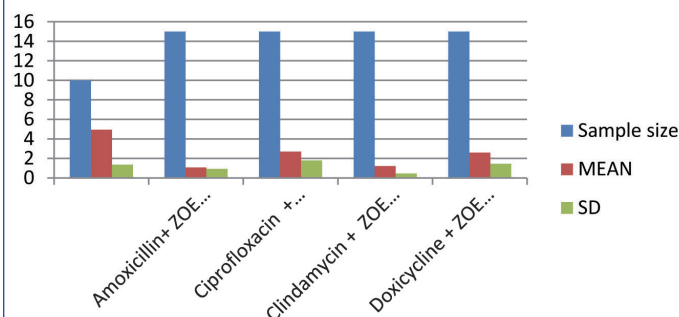
[Table/Fig-1]: Comparison of linear dye penetration between all experimental groups using one-way ANOVA test
Statistically significant at $p < 0.05$; S: Significant

The values obtained were significant between the experimental groups [Table/Fig-2].

Maximum pH of 8.55 was observed with Group II and minimum pH of 4.31 was observed with Group V [Table/Fig-3].

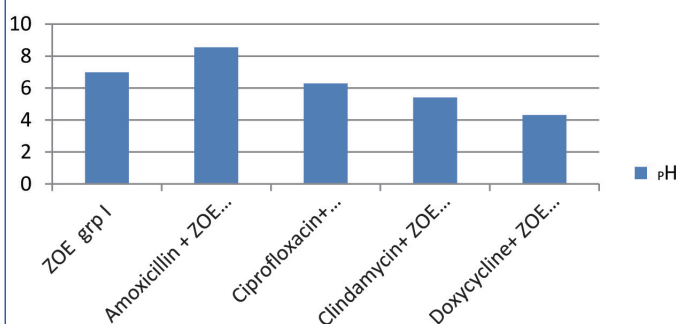
Maximum Viscosity of 2600 cps was observed with Group I and minimum viscosity of 1156 cps was observed with Group II [Table/Fig-4].

Independent sample t-test

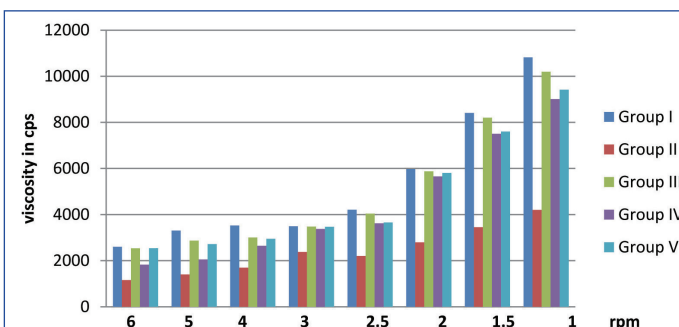


[Table/Fig-2]: Representing comparative linear dye penetration values for all experimental groups using Independent sample t test

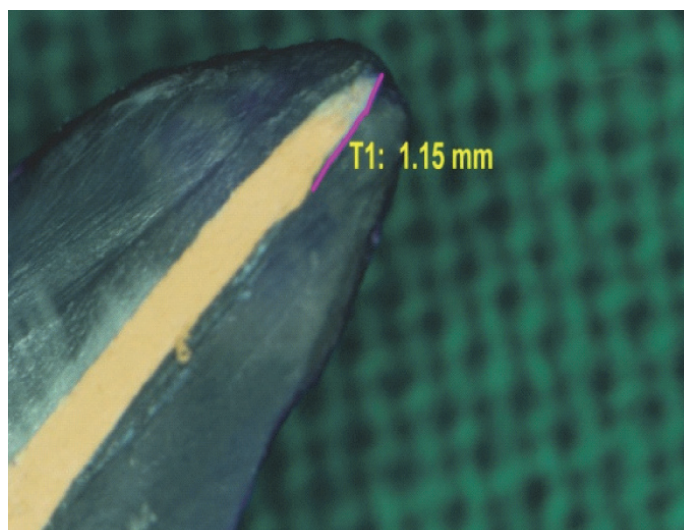
pH



[Table/Fig-3]: pH values of ZOE sealer and various antibiotic combinations



[Table/Fig-4]: Intergroup comparison of viscosities of experimental groups at different rpm



[Table/Fig-5]: Stereomicroscope picture showing linear dye penetration measurement in group II

DISCUSSION

The main aim of root canal treatment is to sanitize and obturate root canal system and to prevent reinfection. With all the advances in endodontic techniques, we still lack a perfect system that can render complete eradication of microorganism from the root canal system. The most common causes are lack of an apical fluid tight seal, percolation of water soluble proteins, enzymes and salts from blood serum into the root canal space back into the periapical tissue which act as irritants and produce periapical inflammation [15]. Sometimes mechanical instrumentation and chemical irrigants may not reach these above specified areas, so the bacteria are left in the obturated root canal. These microorganisms may possess an ability to survive the antimicrobial treatment and have 'persistence' characteristics such as a capacity for starvation survival and an ability to utilize serum-like periapical transudate as a nutritional source [16,17].

The persistent intraradicular or secondary infections, and in some cases extraradicular infections, are the major causes of failure of both poorly treated and well-treated root canals [18]. So there is a need to have an antimicrobial effect by obturating material even after the obturation. So these antimicrobial agents can be incorporated in the gutta-percha or in the sealer or both the substantivity of the tetracycline in medicated gutta-percha is only four hours, so the medicated gutta-percha or the sealer- antibiotic combinations should have long substantivity of antimicrobial effect.

Sealer antibiotic combination proved to be better in eradicating the persistent microbes in the root filled teeth [19]. AH26 sealer-doxycycline combination and AH26 sealer -Amoxicillin combination were effective in killing EF in dentinal tubules [7]. Calcium hydroxide root canal sealers are also effective to some extent in eradicating microbes in root filled teeth. ZOE based, calcium hydroxide based and epoxy resin based root canal sealers, Showed initial microbial inhibition only [18], but it seems equally important to determine the effect over a longer time interval. Sealers, inspite of having broad spectrum antimicrobial activity, should also have good physicochemical properties like less film thickness, more amount of flow, less setting time, least solubility and high pH [20]. The sealers should have less viscosity, so that it flows into areas where the instrumentation and chemical irrigants don't reach, thereby reducing the apical microleakage. Sealer should have high pH, so its alkalinity suppresses the activities of dormant microbes in the obturated canals. It has been proved earlier that addition of antimicrobial agents to the sealers have improved the antimicrobial efficacy significantly. So the question arises whether there is an alteration in the physical properties of ZOE sealer if antibiotics are added. So this study was undertaken to evaluate the sealing ability of root canal sealers, difference in pH and viscosity of sealer when antibiotics were added to them.

Dye penetration technique with 2% methylene blue was used to compare the apical leakage. The ascending order of groups showing microleakage is GROUP II < GROUP IV < GROUP V < GROUP III < GROUP I. The experimental groups showed less leakage when compared to control group. Antibiotic and sealer combination has better sealing ability than sealer when used alone. This is in accordance with the studies where in the sealing ability of sealer with calcium hydroxide was evaluated [21]. The results of pH evaluation showed that the pH values were highest for group II – 8.55 and least for group V - 4.31. The ascending order of groups showing pH is Group V < Group IV < Group III < Group I < Group II.

The ascending order of experimental groups in accordance to their viscosities is Group II < Group IV < Group V < Group III < Group I. This could be because of particle size and type of antibiotics, which was tested with dynamic laser light scattering method. The particle size of amoxicillin, clindamycin and ciprofloxacin are 0.25µm, 0.8µm

& 18.6 µm respectively. For all these antibiotics the particles were spherical and non-agglomerated. Doxycycline has a particle size of 22µm which is crystalline in nature. The highest particle size was with zinc oxide powder i.e., 30–40 µm. PH of amoxicillin and sealer group has shown high pH of 8.55, which reveals that its alkalinity suppress the deleterious actions of microorganisms in the obturated root canals.

CONCLUSION

Within the limits of the study, it is concluded that results of all the experimental groups showed significant difference in apical microleakage when compared to control group. Group II and group IV have shown minimum apical microleakage and viscosity. pH was found to be >7 for Group II and <7 for remaining groups. Amoxicillin + ZOE sealer can improve the success rate of endodontic therapy by enhancing the antimicrobial activity, alkaline environment and reducing apical microleakage.

Further research has to be conducted on post setting solubility, film thickness, dimensional changes and particle size of sealer and antibiotic combination. These parameters must be checked in-vivo conditions to know the changes in the pH and film thickness of the sealer and antibiotic combination. The pH values in this study were noted immediately after fresh mix of sealer and antibiotic, further studies are needed to find whether there is any change in pH at long span after mix and whether there is any chemical reaction between ZOE, resin sealers and antibiotic powder particles.

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