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Research Article

# COMPARATIVE EVALUATION OF ANTIMICROBIAL EFFICACY OF DIFFERENT ROOT CANAL SEALERS AGAINST THE MICROORGANISM *ENTEROCOCCUS FAECALIS* IN AN *EX VIVO* INFECTED ROOT CANAL MODEL BY USING COLONY FORMING UNIT

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## ABSTRACT

**Introduction:** Root canal sealers help to minimize leakage provides antimicrobial activity to reduce the possibility of residual bacteria and to resolve periapical lesion. So the aim of this *ex vivo* study was to compare the antimicrobial activity of five different root canal sealers against *E. faecalis* in an infected root canal model by using Colony Forming Unit (CFU).

**Materials and Methods:** One hundred and twenty single rooted human mandibular premolars were selected. Contamination of the dentine specimens were carried out for 21 days at 37°C. The samples (120) were divided into six groups, Group 1 –Tubliseal, Group 2- Apexitplus, Group 3- Fillapex, Group 4- AH Plus, Group 5- Roekoseal, Group 6- Positive control. Five groups after the incubation with the microorganisms *E. faecalis* was coated with different root canal sealers and obturated using F3 protaper guttapercha point (Dentsply Tulsa dental, USA). Then, approximately 3 mm from the apical portion of the samples were resected horizontally and dentine blocks were obtained (11mm). The root canal filling was removed using a size 2(ISO size 90) and 3(ISO size 110) passo reamers, and the root canals were then enlarged using size 4(ISO size 130mm) to obtain the dentinal shavings from each of the sample and analyzed for CFU.

**Results:** The CFU analysis showed highest antibacterial activity with AH Plus (13700 CFU/ml) and least by Roekoseal (33750 CFU/ml) against *E. faecalis*.

**Conclusion:** It can be concluded that the five different root canal sealers that were used in this study exhibited antimicrobial activity. The maximum antimicrobial activity was achieved AH Plus and Tubliseal. The least antimicrobial activity was achieved with Roekoseal.

**Keywords:** *E. Faecalis*, Root Canal Sealer, Dentine Blocks, Colony Forming Units.

## INTRODUCTION

The main objective of root canal treatment is to eliminate bacteria from the infected root canal and also to prevent reinfection of the root canal. The presence of microbes at the time of obturation can significantly reduce the success rate of endodontic therapy<sup>1,2</sup>. *Enterococcus faecalis* is a gram positive, facultative anaerobe and is a micro-organism which can survive extreme challenges. *E. faecalis* has been shown to be highly resistant once established in the root canal system and is probably the species that can best adapt to and tolerate the ecologically demanding conditions of the filled root canal<sup>3,4,5</sup>.

Root canal sealers are intended to fill the irregularities between the dentinal walls and the core material, as well as accessory and lateral canals. Entombing of bacteria within the root canal system should result in all the remaining bacteria being sealed by the root filling inside the dentinal tubules and lateral canals<sup>6,7</sup>. The use of sealers with antibacterial properties may be advantageous especially in clinical situations of persistent or recurrent infection<sup>6,8</sup>.

Microbial culture techniques have been used for studying endodontic pathogens for many years. It allows for the identification of new species and the quantification of major viable microorganisms. Culture studies are commonly used for

the evaluation of new therapeutic techniques and biomaterials. So this study was undertaken to compare five different root canal sealers against *E. faecalis* in an infected root canal model by using Colony Forming Unit<sup>9,10</sup>.

## MATERIALS AND METHODS

Sixty single rooted human mandibular first premolars were sectioned at or below the cemento-enamel junction with a diamond disc and adjusted to 14mm length. The working length was established approximately 1 mm short of the root length which was confirmed radiographically. All the root canals were instrumented with the Universal Protaper rotary system (Dentsply Maillefer, Switzerland) according to the manufacturer's instructions at a rotational speed of 300 rpm using an endomotor (Endomate NSK, Japan). The teeth were then sterilized by autoclaving for 30 min at 121°C (15lb pressure) and stored in a sterile pouch until use.

### Preparation of inoculums

A suspension of 50µl of *E. faecalis* (ATCC 29212) strain was incubated in 5ml of Trypticase Soy Agar broth (TSA) culture medium (Difco, Sparks, MD, USA) at 37°C in an incubator for 4 hours. The concentration of the inoculation was then adjusted to a level of turbidity 1 according to the McFarland scale (Bio Merieux, Marcy l'Etoile, France), which corresponds to a bacterial load of  $3 \times 10^8$  cells/ml which is referent to an optical density of 550nm. Fifty microlitres of the inoculums containing *E. faecalis* was transferred into each microcentrifuge tube (n=60). Contamination of the dentine specimens was carried out for 21 days at 37°C. Two samples from were then randomly selected for bacterial penetration into the dentinal tubules which was confirmed using scanning electron microscope (SEM). The samples (60) were divided into six groups, Group 1 –Tubliseal, Group 2- Apexitplus, Group 3- Fillapex, Group 4- AH Plus, Group 5- Roekoseal, Group 6- Positive control.

### OBTURATION OF SAMPLES

The different sealers were mixed according to the manufactures instructions and the sealers were applied to the root canals of the respective groups by using a lentulospiral (ISO size 25). Five groups after the incubation with the microorganism *E. faecalis* was obturated using (F3) protaper gutta-percha point (Dentsply Tulsa dental, USA) corresponding to ISO size 30. In each of the study group lateral condensation technique was performed. Auxiliary sizes 20 and 25 cones were condensed until it was not possible to place another accessory cone further than 3 mm into the root canal. In group 6 samples were incubated with *E. faecalis* were dried with paper points and not obturated were used as positive control.

### PREPARATION OF DENTIN BLOCKS

All the samples(n=60) were then placed onto sterile microplates and stored at 37°C and 100% humidity for about 1 week in order to allow the sealers to set<sup>16</sup>. Then, approximately 3 mm from the apical portion of the samples were resected horizontally and dentine blocks were obtained<sup>8</sup> (11mm). The remaining 11mm was used for the evaluation of antimicrobial activity.

## REMOVAL OF ROOT FILLING

The root canal filling was removed using pesos reamers size 2(ISO size 90) and 3(ISO size 110), and the root canals were then enlarged using size 4(ISO size 130) to obtain the dentinal shavings from each of the sample<sup>2</sup>. The dentinal shavings were then collected in micro centrifuge tubes. For each sample collection a sterile pesso reamer was used.

## RESULTS

### STATISTICAL ANALYSIS

Measures of central tendency i.e, Mean and Measures of Dispersion i.e, Standard deviation was calculated for all the parameters. To compare the mean difference of all the parameter between 6 groups of Root Canal Sealers Analysis of Variance (ANOVA) was calculated and to find out the exact significance among those 6 groups of Root Canal Sealers post-hoc test i.e, Bonferroni test was performed. P value < 0.005 was considered significant.

### COLONY FORMING UNIT

When the control group samples were subjected to colony forming unit counts after the inoculation of *E. faecalis* (ATCC 29212), 115000 CFU/ml for *E. faecalis* Colony forming unit counts for all the different group of root canal sealers obtained were compared with the values attained with the control group and was found to be statistically significant. (p< .005)

There was statistically significant difference (p< .005) in the colony forming units of *E. faecalis* (Table 1) in all the different groups of root canal sealers when compared with control group. Statistically significant reduction was seen in Group 4 (AH Plus) 13700CFU/ml followed by Group 1 (Tubliseal) 15000CFU/ml, followed by Group 2 (Apexit Plus) 23750CFU/ml followed by Group 3 (Fillapex) 22250CFU/ml and least reduction was seen in Group 5 (RoekoSeal) 33750CFU/ml.

## DISCUSSION

Root canal treatment aims to eliminate bacteria from the root canal and to promote healing of periapical tissues. Although endodontic procedures can reduce large amount of bacteria, these procedures cannot totally eliminate bacteria from the root canals. Also, part of the root canal space often remains untouched during chemomechanical preparation regardless of the technique and instruments employed which results in root canal treatment failure. To prevent this, root canal sealers are of great importance since they penetrate dentinal tubules and cause microbial inhibition<sup>11</sup>.

The *ex vivo* root canal model which was used with some modification in our study was developed by Haapasalo & Orstavik<sup>2</sup>. This *ex vivo* model of extracted teeth infected with artificial endodontic microflora maintained within the original root canal environment, allowing experimental and bacteriological procedures to be performed in the laboratory without reliance on the patient or the treatment provider<sup>12</sup>.

In this study *E. faecalis* was chosen as the test microorganism because it was recovered in 30–70% of root filled teeth with persistent periapical lesions. It has a special capacity for invasion of dentinal tubules and adhere to the dentine. It also has the property to endure a high alkaline pH of 11.5. The

mechanism of alkaline tolerance of *E. faecalis* is by a functioning cell-wall-associated proton pump, which drives protons into the cell in order to acidify the cytoplasm and this plays an important role in the survival of *E. faecalis* in the highly alkaline environment<sup>13,14</sup>.

In addition to the root canal filling materials, the root canal sealer plays an important role in obturation. The sealer fills all the voids which gutta percha is unable to fill, because of gutta-percha's physical limitations. The sealer acts as a binding agent between the dentine and the core material, which usually is gutta percha. Sealers are usually a mixture that penetrate the dentinal tubules, provides antimicrobial activity and seal the dentinal tubules<sup>15</sup>.

After obturation the samples were placed in the incubator for seven days in order to allow for the sealers to set. After that, apical 3 mm of roots were sectioned and dentine blocks were obtained. The apical 3 mm was eliminated due to the differences arising between the apical delta and the apical lateral canals<sup>8</sup>.

The majority of the clinical studies evaluating the antibacterial effects of root canal sealers have been based on culture techniques. Various methods used are agar disk diffusion test (ADT) direct contact test (DCT) and colony forming unit (CFU). The ADT is a relatively insensitive and semi-quantitative technique which does not distinguish between the bactericidal and bacteriostatic effects of an agent being tested. The results of agar disk diffusion are also highly influenced by the solubility and diffusability of the test agent through the agar, and therefore this test is not suitable to assay water insoluble materials. The direct contact test is a quantitative and reproducible method that simulates the contact of the test microorganism with the endodontic sealers inside the root canal.<sup>10,11</sup>

CFU count was chosen as one of the methods for bacterial evaluation. CFU is a microbiologic sampling technique that can estimate the number of cultivable bacteria and the quantitative analysis of dentine infection can also be done<sup>2,8</sup>.

The anti microbial activity of Tubliseal is primarily due to the presence of eugenol. Eugenol, which is a phenolic compound is bactericidal at relatively high concentrations and it is able to induce cell destruction and inhibit cell growth, respiration and inhibit *E. faecalis* intrinsic mechanism<sup>7,9</sup>. The anti microbial activity of AH Plus is due to the presence of Bisphenol-A-Diglycidylether. Extracts of paste A (containing epoxy resin) and paste B (containing amines) are mixed together whereby the sealer reduced the cell viability<sup>16,17</sup>. This suggests that amines and epoxy resin, can also be toxic and that unpolymerized residues in the mixture might maintain the toxic effect. Also this sealer has a good flow thereby it diffuses into the dentinal tubules and creates microbial inhibition by means of entombment<sup>18,19</sup>.

The incorporation of antibacterial components in root canal sealers may be an important factor in preventing the regrowth of residual bacteria and controlling bacterial re-entry into the root canal system<sup>20</sup>. The MTA based sealer fillapex is more stable providing a constant release of calcium ions and maintains a pH which provides the antibacterial effects and it also has a strong affinity for the release of hydroxyl ions. The initial high pH is 10.3 of fillapex is a significant advantage, as

it is able to exert its effect on the bacterial cell membrane and the protein structure<sup>21,22,23</sup>.

The antimicrobial effect exerted by apexit plus is due to the ionic dissociation of the sealer into calcium ( $\text{Ca}^{2+}$ ) and hydroxyl ( $\text{OH}^-$ ) ions leading to an increase in pH (12.5). A pH greater than 9 reversibly or irreversibly inactivates the microbial cell membrane enzymes, resulting in the loss of biological activity and destruction of the cytoplasmic membrane<sup>24</sup>. The limited antifungal activity of calcium hydroxide sealer might be attributed to a lack of sufficient pH elevation, limited solubility, and diffusibility of calcium hydroxide into dentinal tubules and possibly buffering ions present in the tubules<sup>1,25,26</sup>.

Roekoseal is a silicone based root canal sealer that ensures a homogeneous mix, free of air bubbles and the rheology can be carefully controlled by the addition of the appropriate amount of filler. The small filler particle size ensures that this material has excellent flow properties and can achieve a lesser film thickness, which allows the sealer to flow into tiny crevices and tubules. One concern is that this root-canal sealer has neither the ability to bond to dentine, nor any antibacterial properties<sup>27,28</sup>.

There are some limitations in culture studies such as low sensitivity, misidentification of cultivable strains with ambiguous phenotype, difficulties in detecting culture-difficult species and the inability to grow many species under laboratory artificial conditions. Lack of growth on the plates could be a result of bacteria having changed into a so-called viable but nonculturable (VBNC) state because of the stress caused by the antimicrobial components of the sealers<sup>11</sup>.

Within the root canal system, the antimicrobial activity of root canal sealer is beneficial as it might destroy the remaining microbes that are not destroyed by cleaning, shaping and intracanal medicaments. The escape of sealer into the periapical region while obturation might hinder the regrowth of destructed periodontal ligament fibres and may delay the healing of periapical lesions. Hence the selection of sealer is very important for the healing of periradicular tissues as well as for the antimicrobial activity inside the root canal.

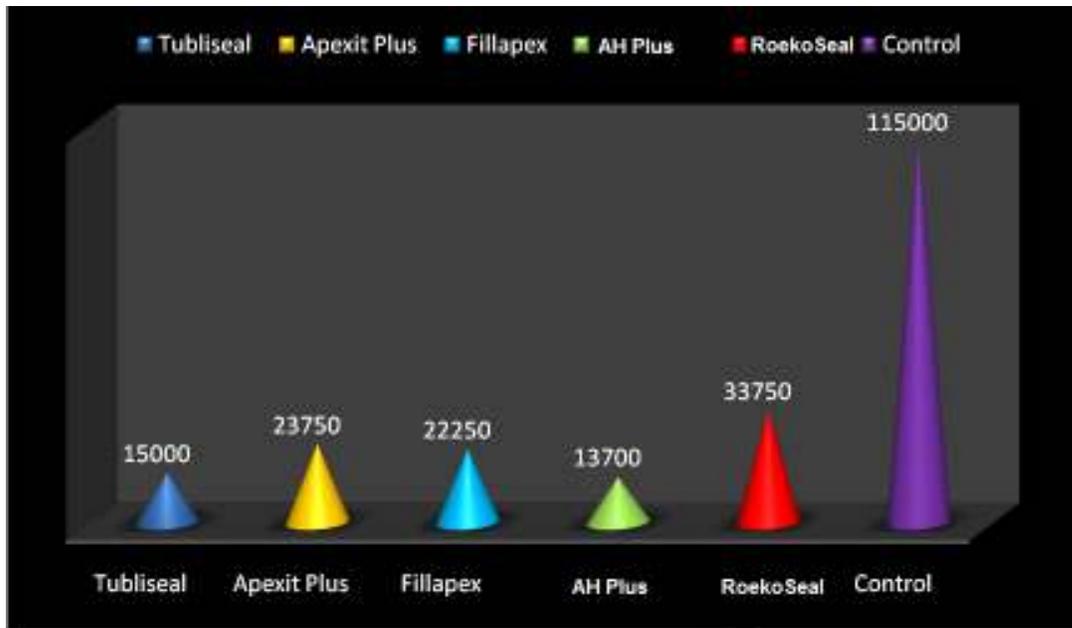
## CONCLUSION

Within the limitations of the study, it can be concluded that the different root canal sealers that were used in this study exhibited antimicrobial activity against *E. faecalis*. The maximum antimicrobial activity against *E. faecalis* was achieved with AH Plus and Tubliseal. Moderate antimicrobial activity against *E. faecalis* was achieved with Fillapex and Apexit plus. The least antimicrobial activity against *E. faecalis* and *C. albicans* was achieved with Roekoseal.

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Graph 1: Comparison of Colony Forming Unit of *E.Faecalis* in Different Groups of Root Canal Sealers

Table 1: Descriptive Analysis (Anova) of Colony Forming Unit of *E.Faecalis* in Different Groups of Root Canal Sealers

Group	Mean ± SD	Mean Square	F	p-value Sig.
Tubliseal	15000±2581.988	1.503E10	779.499	.000
Apexit Plus	23750±1732.050			
Fillapex	22250±1751.983			
AH Plus	13700±1052.774			
Roeko Seal	33750±1354.006			
Control	115000±10000			

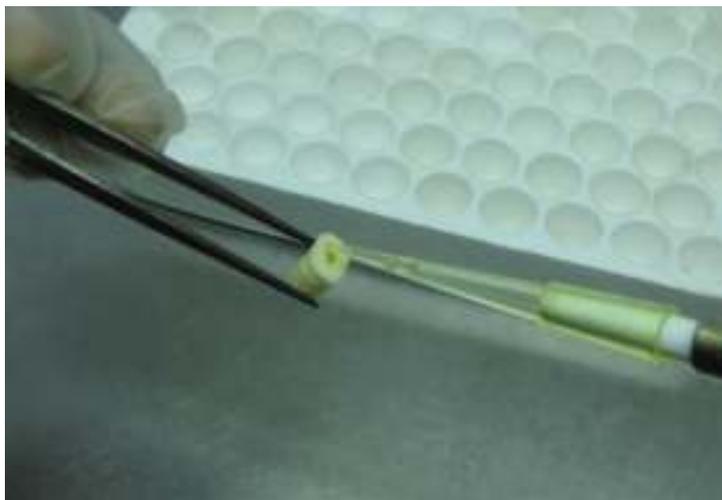


Figure 1: Inoculation of study samples



Figure 2: Dentin Blocks



Figure 3: Colony counter



Figure 4: Dentinal Shavings

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