



Evaluation of Antibacterial Activity of Five Root Canal Sealants against *Enterococcus faecalis* – An *In vitro* Study

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ABSTRACT

Root canal treatment is the process of removing infected, injured or dead pulp from the tooth. Failure, during and after endodontic treatment, is linked to the presence of bacteria in the root canal. The most effective ways to eliminate them are by means of instrumentation and antibacterial irrigation. The use of root canal filling materials with antibacterial activity is considered beneficial in reducing the number of microorganisms and to eradicate the infection. Aim of the present study was to check the antibacterial activity of five root canal sealants against *Enterococcus faecalis*, a predominant bacteria associated in root canal failure. Filter paper discs measuring 6mm diameter were impregnated with different root canal sealers and its activity was tested using disc diffusion technique. The root canal sealers used in this study showed antibacterial activity with Endomethasone showed maximum zone of inhibition of 28 mm and minimum zone size was seen with AH plus of 8mm.

Keywords: Disc diffusion, antibacterial, zone of inhibition, root canal sealer.

INTRODUCTION

Root canal treatment or endodontic treatment, is the process of removing infected, injured or dead pulp from the tooth. The main objective of endodontic treatment is to eliminate bacteria from the root canal system and to prevent them from infecting or re-infecting the root canal or the peri-apical tissues. *Enterococcus faecalis* is a recalcitrant candidate among the many causative agents of failed endodontic treatment. Chronic failure is due to the ability of *E. faecalis* to bind to the collagen of the dentinal tubule and remain viable within the tubules. These microorganisms have the ability to grow even in a low-nutrient environment and can survive in the root canals as a mono infection. Eradication of *E. faecalis* from the root canal with chemo mechanical preparation using disinfecting irrigants and antibacterial dressings is difficult. The most commonly used methods for microbial control include instrumentation, antimicrobial irrigation, intra canal dressing, adequate filling and coronal restoration. The use of root canal filling materials with antibacterial activity is considered beneficial in the effort to further reduce the number of remaining microorganisms and to eradicate the infection. The endodontic sealers have been shown to offer the greatest antimicrobial effects immediately after spatulation, following which there will be a gradual loss of antimicrobial effects over time. The use of sealers with antibacterial properties may be advantageous especially in clinical situations of persistent or recurrent infection.¹⁻³

MATERIALS AND METHODS

In this study, the antimicrobial efficacy of commercially available root canal sealers namely zinc oxide eugenol, AH plus, Endomethasone, Dycal, MTA Fillapex were

evaluated against *Enterococcus faecalis* which is known to be isolated from necrotic pulps and endodontic lesions using the agar well diffusion technique. All sealers were mixed according to manufacturer instructions and loaded on to sterile filter paper discs.

Zinc oxide Eugenol

Zinc oxide eugenol [ZOE] sets because of a combination of physical and chemical reaction, yielding hardening of the mixture due to formation of zinc eugenolate. The presence of free eugenol tend to weaken the set and has increased cytotoxicity. The advantages of ZOE are ease of manipulation, plasticity, radiopaque with some germicidal properties, minimal staining, accepted working time, low setting time in the absence of moisture and good sealing properties. But it causes irritation and not easily absorbed from periapical tissues.

Zinc eugenolate has the disadvantage, however, of being decomposed by water through a continuous loss of the eugenol. This makes ZOE a weak, unstable material and precludes its use in bulk, such as for retrofillings placed apically through a surgical approach.⁴

AH-Plus

AH Plus consists of a paste-paste system, which is delivered in two tubes and in a double barrel syringe. In addition to the di epoxide, the epoxide paste contains radio opaque fillers and aerosil. The amine paste consists of three different types of amines, radio opaque fillers and aerosil. AH Plus is characterised by very good mechanical properties, high radio opacity, little polymerisation shrinkage, low solubility, and, not least, a high degree of stability on storage. The radio opaque fillers used in AH Plus ensure an exceptionally good radio opacity of the material, even when applied in very thin



layers. Tightness and insolubility of the polymerised material are relevant for the function of a root canal sealer.^{5,6}

Endomethasone

Endomethasone is a zinc oxide-eugenol-based (paraformaldehyde containing) sealer that has shown high antibacterial activity. However, its paraformaldehyde content has been the subject of discussion due to the long-lasting inconvenience and disabling complications caused by the use of drugs that contain such a substance. The cytotoxicity of Endomethasone N is approximately 30-folds lower than a formaldehyde containing sealer.⁷

DYCAL (Calcium Hydroxide Containing Sealer)

Calcium hydroxide is used as a root-filling material for stimulation of the periapical tissues in order to maintain health or promote healing and for its antimicrobial effects. Calcium hydroxide is antibacterial depending on the availability of free hydroxyl ions. The alkaline pH of calcium hydroxide neutralizes lactic acid from osteoclasts and prevents dissolution of mineralized components of teeth. This pH also activates alkaline phosphatase that plays an important role in hard tissue formation. Calcium hydroxide activates the calcium-dependent adenosine triphosphatase reaction associated with hard tissue formation. Calcium hydroxide diffuses through dentinal tubules and may communicate with the periodontal ligament space to arrest external root resorption and accelerate healing. Several studies have investigated the relative antimicrobial effects of calcium hydroxide containing sealers and their duration of activity using different bacterial species and different test methods.^{8,9}

MTA Fillapex (Mineral Trioxide Aggregate Based Sealers)

Mineral trioxide aggregate (MTA) is a relatively new and important endodontic material that includes calcium based minerals among of its key ingredients. After hardening, it contains calcium oxide that could react with tissue fluids to form calcium hydroxide. Although MTA is not formulated for use as a sealer around a core material, it is increasingly used particularly to manage cases with open apices. MTA has been thoroughly tested for its solubility, physical properties and its biocompatibility and effects on the periapical tissues. Many of its properties support its use alone as a root canal filling/sealing material. The sealer releases calcium to create an alkaline environment. Use of highly alkaline materials assists in the mineralization of hard tissues, in addition to antimicrobial properties. Based on recent studies, MTA Fillapex has antibacterial activity against *E. faecalis* before it sets.¹⁰⁻¹³

Methodology

The antibacterial effect of the root canal sealers is tested against *Enterococcus faecalis* by disc diffusion technique. The broth culture of the bacterial strain compared to Mac Farland's standard 0.5 was prepared. Lawn culture of the test organism, *Enterococcus faecalis* was made on the

Muller Hinton agar [MHA-Hi media M1084] plates using sterile cotton swab and the plates were dried for 15 minutes. Filter paper discs loaded with different root canal sealers were placed on the agar plates inoculated with the test organism. The inoculated plate with the sealers were kept at room temperature to allow the diffusion of the agents through the agar and were incubated at 37°C overnight and the zone of inhibition of growth was measured in millimeter diameter. All the tests were done in triplicate to minimize the test error.¹⁴⁻¹⁶

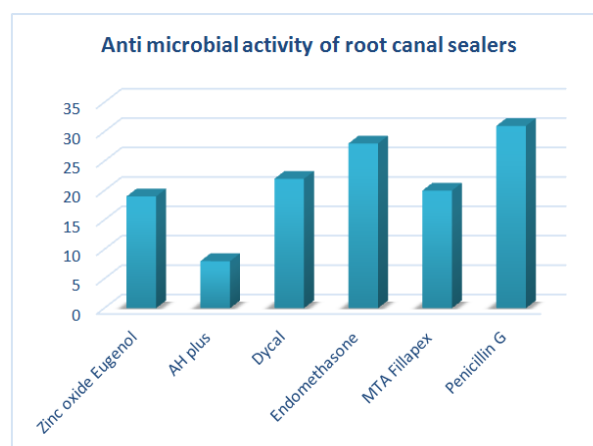
RESULTS AND DISCUSSION

Measuring the Size of the Zone of Inhibition

Growth inhibitory zones around each sealer were evidenced by the lack of bactericidal colonization adjacent to the sealer. The inhibitory zones were measured in millimeters. All the measurements were considered indicative of significant bacterial growth inhibition. Wider zones of inhibition were indicative to greater antimicrobial activity of involving sealers. The results are given in Table 1. Among the sealers tested Endomethasone showed maximum zone of inhibition of 28 mm and minimum zone size was seen with AH plus of 8mm.

Table 1: Antimicrobial Activity of Different Root Canal Sealers against *Enterococcus faecalis*

S. No.	Root Canal Sealants	Zone of Inhibition [In mm diameter]
1	Zinc Oxide Eugenol	19
2	AH plus	8
3	Dycal	22
4	Endomethasone	28
5	MTA Fillapex	20
6	Penicillin G [control]	31



Success of root canal treatment depends upon eliminating microorganism from the root canal system and to prevent reinfection. Thus, endodontic treatment must be carried out under aseptic conditions, using a

powerful irrigant solution, an intra canal medicament, a sealer with antimicrobial activity and an effective coronal seal to prevent coronal micro leakage in order to increase the chances of successful root canal treatment.

The main clinical requirements of a root canal sealer presented in the literature are good tissue compatibility and a lasting tightness of the root canal. Tightness mainly depends on dimensional stability like shrinkage, expansion and solubility as well as adhesion to both dentin and applied cones. In addition an ideal sealant should have antimicrobial activity, good radio opacity and easy application of the material.¹⁷

CONCLUSION

In the present study the antimicrobial activity of 5 sealers were tested against *Enterococcus faecalis*, which is considered to be resistant to endodontic treatment.

All the sealers tested showed different degree of antibacterial activity with endomethasone showing maximum activity against the organism tested. 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.

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