

Comparative Evaluation of Push-out Bond Strength of Three Endodontic Sealers with and without Amoxicillin-An Invitro Study

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ABSTRACT

Introduction: The purpose of this study was to evaluate the sealing ability of three different root canal sealers with and without amoxicillin, using a push-out bond strength test.

Material and Methods: Sixty single-rooted extracted human teeth were used for this study. Each tooth was instrumented and irrigated with 5.25% Sodium hypochlorite (NaOCl) and 17% EDTA. The teeth were then divided into six test groups: Gutta percha (GP)/AH Plus (Dentsply, Germany), GP/AH Plus with 10% amoxicillin (TEVA Pharmaceuticals Sellersville, PA), GP/Pulp Canal Sealer EWT (Extended Working Time, SybronEndo Orange, CA), GP/Pulp Canal Sealer EWT with amoxicillin, GP/Apexit Plus, GP/Apexit Plus (Vivadent, Tulsa,

US) with amoxicillin. After the sealer was set, the entire root was sectioned into 1 mm thick slices. A push-out bond strength test was performed by using a universal testing machine. The Student's t-test was used to compare the sealer bond strength within the specific sealer test groups and within each sealer at apical, middle and coronal root levels.

Results: There was no significant difference between the groups within each sealer ($p > 0.05$) with or without amoxicillin at the same root level.

Conclusion: This study demonstrated that the addition of 10% by weight of amoxicillin does not significantly ($p > 0.05$) change the overall push-out bond strength of three endodontic sealers when compared at the apical, middle, and coronal tooth level.

Keywords: Amoxicillin, AH Plus, Apexit plus, Pulp canal sealer EWT, Push out bond strength, *Enterococcus faecalis*

INTRODUCTION

The major biological aim of endodontic therapy is to eliminate microorganisms by debridement, thorough disinfection and to achieve a fluid tight seal of root canal systems. Bacteria and their by products are considered to be the primary aetiological agents of pulpal necrosis and periapical lesions. The literature suggests that persistent intra-radicular or secondary infections are the major causes of failed root canal treatment [1]. The prognosis of endodontic therapy is significantly influenced by eliminating or reducing the bacterial concentration within the root canal system. Primary endodontic infections are caused by oral microorganisms that may invade a root canal system and establish an infectious process. *Enterococcus faecalis* is the most commonly isolated species being recovered in over one-third of the canals of root-filled teeth with persisting periapical lesions [2]. *Enterococcus faecalis* cells can maintain their viability for extended periods in deprivation and become resistant to UV irradiation, heat, sodium hypochlorite, hydrogen peroxide, ethanol and acid [3]. *Enterococcus faecalis*, unlike others, was found to colonize the root canal system in most cases and to survive without the support of other bacteria [4]. It was shown to invade dentinal tubules and resistant to the anti-microbial effects of calcium hydroxide, that explains its survival in root canal infections [5].

Instrumentation, irrigation and intra-canal medicaments significantly reduce the number of microorganisms inside the infected root canal. However, it is impossible to completely eradicate all microbes from root canal system as bacteria can obstinate in areas such as lateral canals, dentinal tubules and apical ramifications [6]. Consequently, the use of root canal filling materials with anti-microbial activity is considered beneficial in an effort to further reduce the number of remaining microorganisms and to eradicate the infection [7,8].

An ideal endodontic sealer should be biocompatible and dimensionally stable. It should seal well and have a strong long lasting anti-microbial effect which helps to eliminate residual

microorganisms that have survived the chemo mechanical instrumentation and thereby improving the success rate of endodontic treatment [7].

Amoxicillin is a bactericidal, broad-spectrum, beta-lactam antibiotic that inhibits cell-wall synthesis [9]. The bond strength of endodontic sealer to dentine is important in order to maintain the integrity of the root canal seal. Grossman studied the properties of filling materials and suggested that the adhesion of sealers to the root canal walls is important for successful outcome of endodontic treatment [10]. Moreover, Orstavik et al., suggested that a direct relationship can be found between the endodontic sealer bond and micro-leakage [11]. Adhesion of the root canal filling to the dentinal walls seems advantageous for two main reasons. In a static situation, it should eliminate any space that allows percolation of fluids between the filling and the wall. In a dynamic situation, it is needed to resist dislodgment of the filling during subsequent manipulation. Bond strength of Sealers influences the outcome of Endodontic treatment. Literature reveals calcium hydroxide based Sealers to have less bonding to dentin than Eugenol and Resin based Sealers. The addition of amoxicillin to endodontic sealers could change their ability to seal the canal system. The sealing ability of an endodontic sealer is determined by their push-out bond strength. A similar in vitro study conducted on push-out bond strength using Zinc oxide Eugenol, AH Plus and Resilon/RealSeal™ SE based sealers concluded that addition of amoxicillin did not significantly change overall push-out bond strength [12].

The purpose of this in vitro study was to compare the push-out bond strengths of AH Plus, Pulp Canal Sealer EWT and Apexit Plus endodontic sealers with and without amoxicillin. The null hypothesis tested was that there is no significant difference in push-out bond strength within specific sealers with and without Amoxicillin.

MATERIALS AND METHODS

Sealers

Three commonly used sealers were tested. Pulp Canal Sealer EWT (SybronEndo Corporation, Orange, CA) is a zinc oxide eugenol sealer, AH Plus (Dentsply International Inc, York, PA) is an epoxy resin-based sealer, and Apexit plus (Vivadent, Tulsa, US) is a calcium hydroxide based sealer.

Methodology

In this study, sixty freshly extracted human mandibular premolar teeth that were extracted for periodontal and orthodontic reasons were selected.

The teeth were obtained under a protocol approved by the Human Research Ethics Committee. All teeth were initially disinfected by immersion in 5.25% sodium hypochlorite for six hours and followed by storage in sterile saline. Inclusion criteria was single rooted mandibular teeth with single root canal and apical foramen and Root curvatures between 0° to 10° were selected. Radiographs were taken in both mesiodistal and buccolingual directions to rule out any calcifications, resorptions, extra canals and curvature of the root canal. The degree of root curvature was determined by Snider's technique. Teeth with any sort of defects like internal and external resorptions, root caries and open apices were excluded from this study. To maintain a similar root length all teeth were decoronated. Working length was determined by radiographs and by inserting a15 K-file (Dentsply Maillefer) into the canal till the file reaches the apex, which was observed under microscope at 8x magnification. Then the working length was set as 1mm short from the tip of apical foramen.

The teeth were instrumented by using ProTaper (Dentsply Maillefer, Switzerland) rotary files, according to the manufacturer's instructions till F4 and 6 ml of 5.25% sodium hypochlorite was used for irrigation. A final rinse of 6 ml of 17% EDTA over 1 minute time period was accomplished. The prepared root canals were then dried with paper points and the teeth were randomly assigned to one of the six obturation groups:

- Group 1: Gutta percha and AH Plus
- Group 1A: Gutta percha and AH Plus with Amoxicillin
- Group 2: Gutta percha and Pulp Canal Sealer EWT
- Group 2A: Gutta percha and Pulp Canal Sealer EWT with Amoxicillin
- Group 3: Gutta percha and Apexit Plus
- Group 3A: Gutta percha and Apexit Plus with Amoxicillin

Each obturation group consisted of ten samples where obturation was done by using the specified gutta-percha and sealer combination. In Group 1, 2, 3 plain sealer was mixed and used according to the manufacturer's instructions. In group 1A, 2A, 3A - The sealers were mixed with 10% by weight of crushed amoxicillin. The coronal aspect of each root was sealed with 1 mm of Fuji IX glass ionomer cement (GC Corporation, Tokyo, Japan).

Manipulation of Sealer and obturation of teeth were carried out by a single operator to minimize the chances of errors as these procedures are technique sensitive. No efforts were made to evaluate the film thickness and solubility of the Sealers.

The obturated teeth were stored at 37°C in 100% humidity for 14 days to allow for the sealer to set. Each tooth was sectioned perpendicular to the long axis into 9 slices of 1-mm thick (i.e., three slices each from apical, middle, and coronal thirds) by using an IsoMet saw (Buehler Ltd, USA) with water coolant. Slices from all six groups were collected and slices containing filling material of a noncircular shape were discarded to avoid non-uniform stress distribution during testing to avoid inaccurate measurements. Plunger that most closely matched the diameter of the filling material, without contacting dentin was connected to a universal

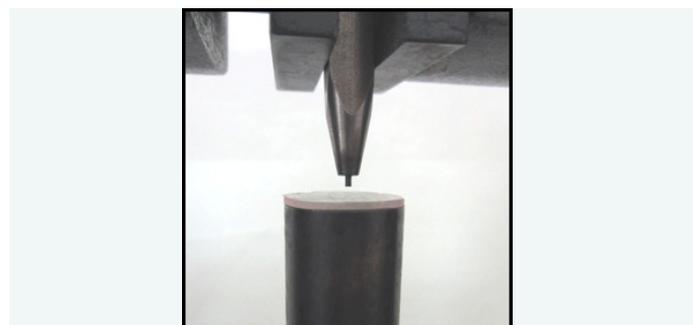
testing machine (5500 Instron Corporation, USA) [Table/Fig-1]. A vertical load was applied to the obturation material in an apical to coronal direction at the rate of 0.5 mm/min.

Failure of bond was determined when a sharp decline was observed on a load versus time curve plotted in real time. The bond strength, expressed in MPa, was calculated by dividing the maximum load in Newton's by the area of the bonded interface. The area of the bonded interface was calculated using the formula, $area = 2\pi r h$, where π is the constant 3.14, and r and h are the measured radius and height in millimetres of the filling material.

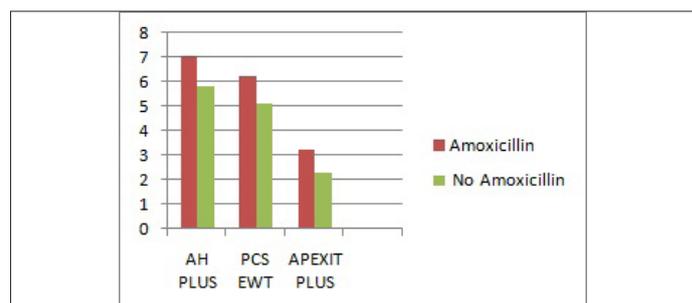
RESULTS

The testing data for push out bond strength were analyzed statistically by using student t-test. The t-test was used to determine an overall difference in bond strength between matched obturation groups (Group 1 and 1A, Group 2 and 2A, and Group 3 and 3A) with and without amoxicillin, regardless of tooth level. Analysis was also completed to compare matched obturation groups with and without amoxicillin at the apical, middle, and coronal levels of the root using the independent sample t-test.

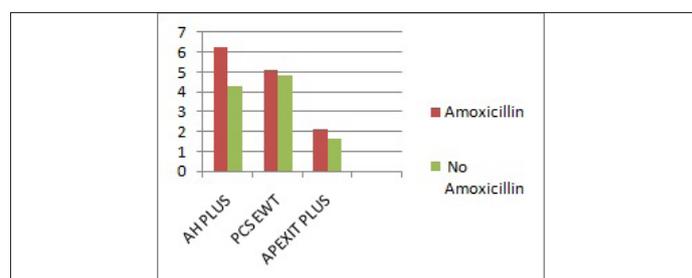
Ten teeth were prepared for each test group. The mean push out bond strengths of each group were: Group 1: 7.66±1.25 MPa; Group 1A: 5.34±2.50 MPa; Group 2: 6.13±1.01 MPa; Group 2A: 4.00±2.03 MPa; Group 3: 3.49±1.25 MPa; and, Group 3A: 2.18±1.08 MPa. There was no significant difference in push out bond strength between the test groups within each sealer, Group 1 vs Group 1A, Group 2 vs Group 2A, Group 3 vs Group 3A ($p > 0.05$) and within each sealer with and without amoxicillin at the same root level (apical, middle, coronal) ($p > 0.05$). Results presented in [Table/Fig-2A,B,C].



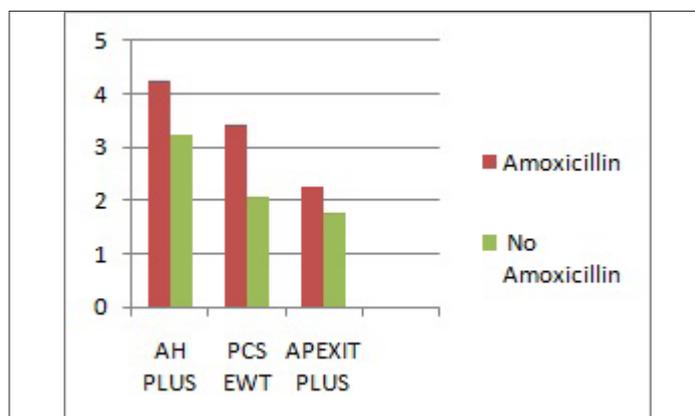
[Table/Fig-1]: Universal Testing Machine



[Table/Fig-2A]: Push out bond strength (MPa) mean values of all groups at apical, middle and coronal level. (A) Push out bond strength (MPa) mean values of all groups at apical level



[Table/Fig-2B]: Push out bond strength (MPa) mean values of all groups at middle level



[Table/Fig-2C]: Push out bond strength (MPa) mean values of all groups at coronal level

DISCUSSION

The absence of microorganisms in the root canal system is a fundamental factor for the prognosis of endodontic treatment. Prognosis is the forecast of the course of a disease. To achieve this goal, the filling material should allow sealing of the root canal at both the root apex and the crown [13]. Microorganisms are the major aetiological agents responsible for pulpal and periapical pathology. Failure to prevent percolation of fluids and to eliminate microorganisms and their by-products might result in persistent irritation and impaired healing.

Enterococcus faecalis, facultative anaerobic gram positive cocci, has been implicated in persistent root canal infections. It causes up to 90% of enterococcal endodontic infections. Although they make up only a small proportion of the initial flora of untreated teeth with necrotic pulps, *Enterococcus faecalis* have been frequently found in obturated root canals exhibiting signs of chronic apical periodontitis and it can survive in very harsh environments [14,15]. It is able to form a biofilm that helps it to resist destruction by enabling the bacteria to become more resistant to phagocytosis, antibodies and anti-microbials than non biofilm producing organisms.

Obturation is achieved with the association of a solid filling material such as guttapercha and a root canal sealer. Ideally, one of the key roles of the sealer is to aggregate the root filling material, maintain it as compact mass with no gaps which adheres to the canal walls and provides a single block configuration that seals hermetically the root canal space [16]. This adhesion process involves mechanical forces that yield the intertwining of the material with the dentin structures and may result in a greater sealing ability, thus reducing the risk of root canal micro leakage and maintaining a cohesive filling mass.

To prevent new bacterial growth, obturation materials and sealers should have anti-microbial properties which upon contact with microbes and biofilms, will prevent re-infection of root canal system. Ideally, over time these sealers should be able to maintain this effect. Adding antibiotics to a sealer can enhance their anti-microbial effect and could provide an important advantage in reducing the critical concentration of microbes necessary for a favourable host response.

Baer et al., has found that local application of antibiotics by a sealer is more effective mode for delivery in Endodontics [17]. Amoxicillin, Clindamycin and Moxifloxacin are the antibiotics of choice for the treatment of odontogenic infections. In this study Amoxicillin was used with the sealer as an anti-microbial agent.

Amoxicillin, an acid stable semi-synthetic drug belongs to a class of antibiotics called the Penicillin's. It is shown to be effective against a wide range of infections caused by gram positive and gram negative bacteria in both human and animals [18].

Endodontic sealers with added amoxicillin showed inhibition of bacterial cell growth initially, but also demonstrated inhibition after 7 days of sealer set [19].

An antibiotic enhanced sealer disrupts the microbial environment and maintains bactericidal properties beyond its setting time which is monumental in the outcome of initial endodontic therapy and in preventing re-infection. But, this addition of antibiotic to the sealer might disrupt the sealing ability of the root canal sealer. In this study, influence of Amoxicillin on bond strength of sealers was evaluated.

AH Plus is epoxy resin based endodontic sealers which can be used with gutta-percha to obtain a three dimensional filling. Epoxy resin sealers are popular because of their apical seal and their micro-retention to root dentin. Epoxy resin-based sealers penetrate deeper into the micro irregularities owing to its flowability and long polymerization time, which contribute to enhancing the mechanical interlocking between sealer and dentine [20]. Swelling of the epoxy resin component of AH Plus after water sorption may also have increased its resistance against dislodgement [21]. These properties lead to greater intertwining of the sealer with dentin structure, which, together with the cohesion among the cement molecules provides greater adhesiveness and resistance to dislodgment from dentin [22,23].

Apexit Plus is a calcium hydroxide based sealer in which Calcium hydroxide does not bond to dentin. The anti-microbial effect of this sealer depends on the dissociation of calcium hydroxide into Ca^{++} and OH^{-} ions which raises the pH to above 12.5 [24]. A study on anti-microbial activity of sealers showed Apexit plus to be having lowest anti-bacterial effect on *enterococcus faecalis* [7].

Zinc Oxide Eugenol (ZnOE) based root canal sealers have been traditionally used in the obturation of root canal system. These sealers produced the largest inhibitory zones against all microorganisms. In ZnOE sealers, Eugenol is a potent anti-microbial agent and therefore, it may be attributable to the free eugenol released from the set materials [25,26]. Stevens et al., found that a final rinse with 95% ethanol increased the depth of penetration of a ZnOE based sealers and significantly reduced micro leakage [27]. Tests on dentinal tubule penetration have shown calcium hydroxide based sealers to be less effective in killing bacteria when compared to resin and eugenol based sealers [28,29]. Direct contact tests of sealers and microorganisms concluded calcium hydroxide sealers to be having mild anti-microbial effect over a short duration [30,31].

In another study, calcium hydroxide based sealers showed poor dentin adhesion [32-35]. One study showed calcium hydroxide based sealer (Apexit) inhibited Gram negative bacteria more effectively than gram positive bacteria [36]. Owing to these shortcomings adding Amoxicillin a Bactericidal, Broad spectrum, beta-lactum antibiotic to calcium hydroxide sealers should have synergistic effect and at the same time should not have negative effect on bond strength of the Sealer at the expense of anti-microbial activity.

A study on bond strength of different endodontic sealers to dentin revealed that, irrigation with 2.5% NaOCl and 17% EDTA have positive effect on push out bond strength [37]. A study conducted on effect of different irrigating solutions and Endodontic Sealers on Bond Strength of dentin concluded that the number and size of defects within the Sealer or at the Sealer dentin interface has negative influence on the bond strength [38].

Various methods have been used to measure bond strength in root dentine which includes pull-out tests, micro tensile tests and push-out techniques. The push-out bond strength test is one way to evaluate the effectiveness of an endodontic obturation material or technique. In this study, this test was done to evaluate the dentine bond strengths of root canal sealers and was based on a

similar study [12] because of its accuracy, reliability, effectiveness, easy reproducibility and ability to detect even lower values. The results after evaluation corroborated with the results of previously done studies [12]. These results showed that in spite of poor dentin adhesion of calcium hydroxide based Sealers, when compared to Eugenol and Resin based Sealers, adding amoxicillin to the sealer did not significantly change the push-out bond strength.

The results of this study are in agreement with the results of other studies comparing the bond strength of gutta percha to dentin using different sealers. The current study made no attempt to compare push-out bond strength between different sealers in both the groups (with amoxicillin and without amoxicillin). Based upon the results, further studies have to be done for the efficacy of Sealers with adding Amoxicillin.

In conclusion, this study demonstrates that AH plus, Apexit plus, Pulp canal sealer EWT have showed no significant ($p > 0.05$) difference in push-out bond strength when mixed with amoxicillin. The results of the present study suggested that addition of amoxicillin to the endodontic sealers has no effect on push out bond strength. Therefore, the null hypothesis is accepted, and the alternative hypothesis is rejected.

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