Journal of Research in Medical and Dental Sciences
2018, Volume 6, Issue 1, Page No: 413-417

Available Online at: www.jrmds.in
eISSN No. 2347-2367: pISSN No. 2347-2545

Comparison of Antibacterial Activity of Three Endodontic Sealers against *Enterococcus faecalis*

Salma omidi[1], Narjes Hoshyari[1], Ali Reza Mirzadeh[2], Mehran Ebrahimzadeh Hassanabadi[3], Mohammad Ahajan[4], Jamshid Yazdani Charati[5], Azam Haddadi Kohsar[1*]

[1]Assistant Professor of Endodontics, Dental Faculty, Mazandaran University of Medical Sciences, Sari, Iran
[2]Dentist, Private Practice
[3]Dental Student, Faculty of Dentistry, Student Research Committee, Mazandaran University of Medical Sciences, Sari, Iran
[4]Department of Microbiology, Faculty of Para Medicine, Mazandaran University of Medical Sciences, Sari, Iran
[5]Associate Professor, Biostatical, Faculty of School of health, Mazandaran University of Medical Sciences, Sari, Iran

DOI: 10.5455/jrmds.20186167

ABSTRACT

one of the important property of endodontic sealer is antibacterial activity. This study aimed to compare the antibacterial properties of three common and widely used sealers: MTA-FillApex, AH Plus, and AH26 against Enterococcus faecalis. In this study, 25 Blood Agar plates were prepared, in each of which 5 wells were devised. In three wells, three sealers of MTA-FillApex, AH Plus, and AH26 were placed. Ampicillin was poured into the fourth well as the positive control group. Finally, distilled water was poured into the fifth well as the negative control group. Concurrently, 1/10 ml of Enterococcus faecalis bacteria suspension with a concentration of 0.5 McFarland was cultivated on the plates. Following 48 hours of incubation, the diameter of inhibition zone around the wells was measured. Finally, the obtained results were analyzed by one-way ANOVA, fisher LSD, Fisher individual 95% Cl. The results of our study on 25 plates after 48 h indicated AH26 sealer with the mean zone of inhibition diameter of 16.44 mm had the greatest antibacterial effects. AH26 was followed by MTA-FillApex with the mean zone of inhibition diameter of 15.44 mm. Finally, the weakest antibacterial effect was related to AH Plus sealer, with the mean zone of inhibition diameter of 10.2 (p<0.1000). According to our study, AH26 sealer has the greatest power and antibacterial properties against Enterococcus faecalis bacteria, followed by MTA-FillApex, and AH plus (as the weakest antibacterial agent against the mentioned bacteria).

Key words: Sealer, Antibacterial Properties, *Enterococcus faecalis*, MTA-Fillapex, AH plus, AH26

HOW TO CITE THIS ARTICLE: Salma omidi1, Narjes Hoshyari, Ali Reza Mirzadeh, Mehran Ebrahimzadeh Hassanabadi, Mohammad Ahajan, Jamshid Yazdani Charati, Azam Haddadi Kohsar, Comparison of Antibacterial Activity of Three Endodontic Sealers against *Enterococcus faecalis*, J Res Med Dent Sci, 2018, 6 (1): 413-417, DOI: 10.5455/jrmds.20186167

Corresponding author: Azam Haddadi Kohsar
e-mail✉ haddadi_azam@yahoo.com
Received: 09/09/2017
Accepted: 20/12/2017

INTRODUCTION

The remaining of microorganisms and their byproducts in the root canal system following primary root treatment has been considered as a primary cause of failure in root treatment [1]. Success in sealing is directly related to removal of microorganisms and their productions through mechanical cleaning andshaping, washing with antibacterial materials, use of antibacterial deressing between session when necessary such as calcium hydroxide, and then canal filling [2, 3]. However, this process does not fully sterilize the inside of canals[4].

Therefore, growth and proliferation of microorganism remained inside the root canal may degrade the tissues around the root, causing periapical lesions [5, 6].

In addition, none of the dentistry materials provide complete sealing with the cavity walls, and micron spaces always remain within the distance between the material and cavity wall. Therefore Microorganisms can penetrate through this space, highlighting the necessity of having antibacterial properties for these materials[7].

One of the microorganisms which are most abundant in failed treatments is *Enterococcus faecalis* [8-11].

Enterococcus faecalis is a gram-positive anaerobic bacteria, found in 38% of failed root treatments. Its ability in tolerating unsuitable environmental conditions, which is a result of high tolerance in alkaline environments and the tubular attack potential cause this bacteria to be resistant to intra canal medications [6, 11].

Gutta-percha is the main substance for filling root canals. As this material is not able to attach to canal walls, to fill the remaining space between Gutta-percha and canal walls, various sealers are used. Thus, presence of a sealer is necessary for sealing the root canal system [12].

Sealer AH26 (Dentsply, Tulsa Dental, Tulsa, OK, USA) is widely used as sealer. A freshly prepared AH26 has high toxicity, but over time, as the compound hardens, this toxicity declines, and after 24 h, it has the minimum tissue toxicity among endodontic sealers. Liberation of trace amounts of formaldehyde during the chemical hardening process of this sealer is a major cause of its toxicity [13]. Hexamethylene teteramine in this sealer is responsible for the antibacterial properties of this sealer [14].

MTA FillApex (Angelus, Londrina, PR, Brazil) is an MTA-based sealer, which is a suitable material to treat root، especially root treatment in teeth with immature root [15]. When MTA-Fillapex is mixed with water, the calcium oxide inside it reacts with water and produces calcium hydroxide. This material causes elevation of pH and enhanced antibacterial properties of MTA-Fillapex [16, 17].

AH plus (Densply, Tulsa Dental, Tulsa, OK, USA) improved properties including liberation of formaldehyde during chemical hardening process of this sealer. AH Plus has a suitable flow and well seals the dentine. It has also a very high opacity (greater than that of AH26), which does not affect the teeth color [18].

As one of the most important factors in success of root treatment is the antimicrobial properties of sealers [14], in this study, this property was compared across AH plus, MTA-Fillapex, and AH26 sealers.

MATERIALS AND METHODS

The research protocol was approved by the Ethics committee of Mazandaran University of Medical Sciences, sari, Iran (IR.Mazums . REC.95.2687).

This type of study is experimental, in which endodontic sealers of MTA-Fillapex, AHplus, and AH26 were examined and compared through Agar Diffusion Test (ADT) method. First, streptococcus faecalis bacteria prepared by Institute Pasteur with ATCC Number 1394 were cultivated out of standard strain samples (Asr E Engelab Co., Tehran) in Broth Hinton Muller culture medium. It was then cultivated in Agar blood culture medium after 6 h at 37°C, and then incubated at 37°C for 24 h. After the incibarion, 3-4 colonies were taken by sterile anas. Then, in a test tube containing Broth Hinton Muller, we prepared a turbidity of 0.5 McFarland. In the plate of this culture medium, 4 wells were developed with a diameter of 6 mm, and then numbered. Thereafter, as much as 0.5 McFarland was extracted from the culture-containing tube using a sterilie swab and cultured as lawn. After that, MTA-FillApex, AH plus, and AH26 were placed in to wells and distilled water were inoculated as negative control in the wells. Then, a 30-micro –gram ampicillin disc (Sobhan Co., Tehran, Iran) was placed across the plate as positive control. The plates were exposed to 37°C for 24 h, and then the zone of inhibition was measured by a ruler in terms of mm. Each of the samples was replicated three times. Thereafter, all numbers were examined by Minitab 17, and then analyzed by one way ANOVA, fisher LSD, and Fisher Individual 95% Cl.

RESULTS

The results of our study on 25 plates after 48 h indicated that considering *Enterococcus faecalis* bacteria, AH26 sealer had the greatest antibacterial effect with the mean zone of inhibition diameter of 16.55 mm. It was followed by MTA-Fillapex with the mean zone of inhibition

diameter of 15.44. Finally, the weakest antibacterial properties were related to AH plus (mean zone of inhibition diameter = 10.2) (p<0.1000).

Table 1: Mean (SD)zone of inhibition diameter of *Enterococcus fecalis* with regard to the type of sealers and control group

Group	Mean ± SD	Ci%95	P-Value
AH26	16.44±1.557	(15.790;17.090)	0.000
MTA-Fillapex	15.44±2.123	(14.790;16.090)	
AHpluse	10.200±1.291	(9.550;10.850)	
Ampicillin	17.840 ±1.463	(17.190;18.490)	

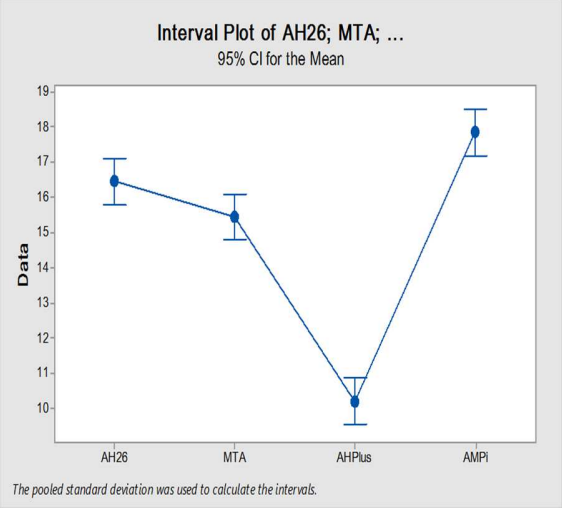

Diagram 1: The scale of power of AH26, MTA, AH plis, Ampicillin, with confidence interval of 95%

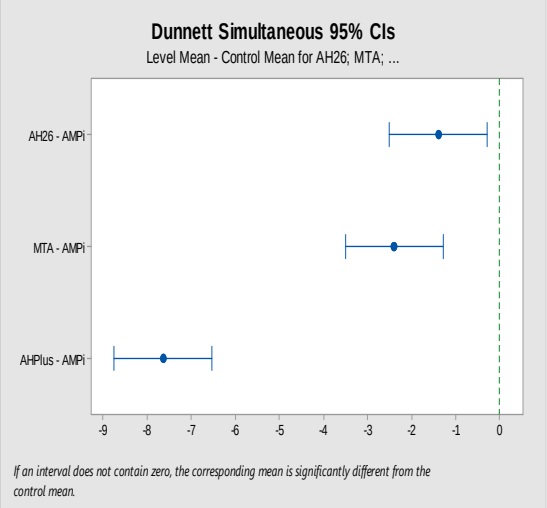

Diagram 2. The scale of three sealers was performed with the control group (ampicillin). According to the diagram, AH26 and MTA have the minimum difference, while AH plus has the greatest difference with the positive control group.

DISCUSSION

Sealers develop a firm seal against permeation of liquids, and the primary goal of using seals and endodontic cements is to fill the space among the Gutta-percha cone and the canal walls or the spaces among the Gutta-percha cones proper. As a lubricator, sealers also facilitate entrance of the main Gutta-percha mixture into the root canal. In addition, sealers are able to block delicate secondary canals [19].

Due to the prevalence of obligate and facultative anaerobic bacteria in unsuccessful endodontic treatments, using sealers with bactericidal activity can control the infection caused by these micro-organisms. The microorganism tested in this study (*Enterococcus faecalis*) is an facultative anaerobic microorganism, which is the most common microorganism in resistant root canal infections and retreatment apical peiodontitist [17, 20, 21].

Agar diffusion method is widely in use for research on antibacterial activity of dental and medical materials [21-23]. This method allows sealers to be in direct contact with microorganisms and sealers have the ability eliminate bacteria in areas such as root canal system. there are factors that affect the results of this method including the necessity of establishing the same contact surface between bacteria and sealers, an equal bacterial particle size, viscosity of agar gel, temperature, and ionic concentration of culture medium and time period for all studied specimens [23, 24].

As the most important property of sealers is being bacteriostatic or at least not causing bacterial growth [14], in this study, attempts were made to compare the power and antibacterial properties of three of the most well-known and widely used sealers available against enterococcus bacteria.

According to our study using diffusion agar test, AH26 sealer has the greatest power and antibacterial properties, followed by MTA-FillApex, and AH plus as the weakest antibacterial agent against the mentioned bacteria.

In the study by Ehsani *et al*., using diffusion agar test, it was found that the antibacterial activity of AH26 sealer on *Enterococcus faecalis* and lactobacillus is greater than that of the MTA Fillapex [25], which is in line with our findings.

Tanomaru *et al*., studied the antimicrobial effects of MTA-Fillapex and AH26. They observed that AH26 had greater antimicrobial activity in comparison to MTA [26]. In our study also AH26 revealed a greater antimicrobial activity compared to MTA FillApex,.

Jafari *et al*., examined the antibacterial effect of AH26 and MTA Fillapex against *Staphylococcus aereus*, *Enterococcus faecalis*, *Lactobacillus acidophile*, and *Lactobacillus aereus*. They found that the antibacterial properties of AH26 were greater than those of MTA Fillapex [17]. In our study also AH26 had a greater antimicrobial activity than MTA FillApex,.

Using Agar Diffusion test, Madani Z *et al*., indicated that the antibacterial activity of MTA Fillapex on *Enterococcus faecalis* is greater than that of AH26, which is incongruent with our results. This discrepancy can be due to different genetic of the enterococcus faeclis samples assessed in the present study [27].

Yasuda *et al*., explored the antimicrobial effects of AH plus, SealApex, MTA, and Super Band sealers on Candida Albicans, staphylococcus Aereus, *Enterococcus faecalis*, streptococcus mutans, and *Streptococcus sanguis* bacteria. AH plus indicated the maximum antibacterial activity, which is not in line with our findings. This mismatch can be due to different samples evaluated in their study [28].

CONCLUSION

In this study, AH26 sealer had greater antibacterial properties against *Enterococcus faecalis*, compared to MTA Fillapex and AHPluse.

Acknowledgments

The authors would like to thank the Mazandaran University of Medical Sciences for their supports.

REFERENCES

1. Al-Hezaimi K, Naghshbandi J, Oglesby S, Simon JH, Rotstein I. Comparison of antifungal activity of white-colored and gray-colored mineral trioxide aggregate (MTA) at similar concentrations against Candida albicans. Journal of Endodontics. 2006; 32(4):365-67.
2. Reit C, Dahlén G. Decision making analysis of endodontic treatment strategies in teeth with apical periodontitis. International Endodontic Journal. 1988; 21(5):291-99.
3. Sundqvist G, Figdor D, Persson S, Sjögren U. Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 1998; 85(1):86-93.
4. Abdulkader A, Duguid R, Saunders E. The antimicrobial activity of endodontic sealers to anaerobic bacteria. International Endodontic Journal. 1996; 29(4):280-83.
5. Torabinejad M SS. Pulp and periapical pathosis. Endodontics Principles and practice fourth ed. St Louis: Saunders/Elsevier, 2009: 49-67.
6. Kangarlou A, Neshandar R, Matini N, Dianat O. Antibacterial efficacy of AH Plus and AH26 sealers mixed with amoxicillin, triple antibiotic paste and nanosilver. Journal of Dental Research, Dental Clinics, Dental Prospects. 2016; 10(4):220.
7. Kakehashi S, Stanley H, Fitzgerald R. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surgery, Oral Medicine, Oral Pathology. 1965; 20(3):340-49.
8. Tabrizizadeh M, Rasti M, Ayatollahi F, Mossadegh MH, Zandi H, Dehghan F, *et al.* Antimicrobial Activity of Calcium Hydroxide and Betamethasone on Enterococcus faecalis; An in vitro Assessment. Iranian endodontic journal. 2015;10(3):184-87.
9. Bhandari S, Ashwini T, Patil CR. An in vitro evaluation of antimicrobial efficacy of 2% chlorhexidine gel, propolis and calcium hydroxide against Enterococcus faecalis in human root dentin. Journal of Clinical and Diagnostic Research: JCDR. 2014; 8(11):ZC60.
10. Prabhakar A, Taur S, Hadakar S, Sugandhan S. Comparison of antibacterial efficacy of calcium hydroxide paste, 2% chlorhexidine gel and turmeric extract as an intracanal medicament and their effect on microhardness of root dentin: an in vitro study. International Journal of Clinical Pediatric Dentistry. 2013; 6(3):171-77.

11. Mozayeni MA, Haeri A, Dianat O, Jafari AR. Antimicrobial effects of four intracanal medicaments on enterococcus faecalis: an in vitro study. Iranian endodontic journal. 2014;9(3):195-98.
12. Tyagi S, Mishra P, Tyagi P. Evolution of root canal sealers: An insight story. European Journal of General Dentistry. 2013;2(3):199-218.
13. Hargreaves KM, Berman LH. Cohen's pathways of the pulp. Elsevier Health Sciences, 2015.
14. Van Noort R. Introduction to Dental Materials4: Introduction to Dental Materials. Elsevier Health Sciences, 2013.
15. Salles LP, Gomes-Cornélio AL, Guimaraes FC, Herrera BS, Bao SN, Rossa-Junior C, et al. Mineral trioxide aggregate–based endodontic sealer stimulates hydroxyapatite nucleation in human osteoblast-like cell culture. Journal of Endodontics. 2012; 38(7):971-76.
16. Baek S-H, Plenk H, Kim S. Periapical tissue responses and cementum regeneration with amalgam, SuperEBA, and MTA as root-end filling materials. Journal of endodontics. 2005; 31(6):444-49.
17. Jafari F, Kafil HS, Jafari S, Aghazadeh M, Momeni T. Antibacterial Activity of MTA Fillapex and AH 26 Root Canal Sealers at Different Time Intervals. Iranian Endodontic Journal. 2016; 11(3):192-97.
18. Miletić I, Prpić-Mehičić G, Maršan T, Tambić-Andrašević A, Pleško S, Karlović Z, et al. Bacterial and fungal microleakage of AH26 and AH Plus root canal sealers. International Endodontic Journal. 2002; 35(5):428-32.
19. Copeland HI, Brauer GM, Forziati A. The setting mechanism of zinc oxide and eugenol mixtures. J Dent Res. 1955; 34:740.
20. Zhang H, Shen Y, Ruse ND, Haapasalo M. Antibacterial activity of endodontic sealers by modified direct contact test against Enterococcus faecalis. Journal of Endodontics. 2009; 35(7):1051-55.
21. AlShwaimi E, Bogari D, Ajaj R, Al-Shahrani S, Almas K, Majeed A. In vitro antimicrobial effectiveness of root canal sealers against enterococcus faecalis: A Systematic Review. Journal of Endodontics. 2016; 42(11):1588-97.
22. Eldeniz AU, Erdemir A, Hadimli HH, Belli S, Erganis O. Assessment of antibacterial activity of EndoREZ. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics. 2006; 102(1):119-26.
23. Gomes BPFdA, Pedroso JA, Jacinto RC, Vianna ME, Ferraz CCR, Zaia AA, et al. In vitro evaluation of the antimicrobial activity of five root canal sealers. Brazilian Dental Journal. 2004; 15(1):30-35.
24. Forbes B. SD WS. Diagnostic microbiology. 12th, editor: Mosby, 2007.
25. Ehsani M, Adibi A, Moosavi E, Dehghani A, Khafri S, Adibi E. Antimicrobial activity of three different endodontic sealers on the enterococcus faecalis and lactobacillus (in vitro). Caspian Journal of Dental Research. 2013; 2(2):8-14.
26. Tanomaru-Filho M, Tanomaru JM, Barros DB, Watanabe E, Ito IY. In vitro antimicrobial activity of endodontic sealers, MTA-based cements and Portland cement. Journal of Oral Science. 2007; 49(1):41-45.
27. Madani Z, Sefidgar S, Rashed AM, Zabihi E, Mesgarani A, Bijani A, et al. Comparative evaluation of antimicrobial activity of two root canal sealers: MTA Fillapex and AH 26. Minerva Stomatologica. 2014; 63(7-8):267-72.
28. Yasuda Y, Kamaguchi A, Saito T. In vitro evaluation of the antimicrobial activity of a new resin-based endodontic sealer against endodontic pathogens. Journal of Oral Science. 2008; 50(3):309-13.