EVALUATION OF ANTI E-FAECALIS EFFICACY AND PENETRATION DEPTH OF CURCUMIN LONGA MODIFIED SEALER-AN IN VITRO CLSM STUDY

Dissertation submitted to

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY

In partial fulfillment for the degree of

MASTER OF DENTAL SURGERY

BRANCH – IV

CONSERVATIVE DENTISTRY AND ENDODONTICS

APRIL 2017

ENDORSEMENT BY THE H.O.D. PRINCIPAL / THE HEAD OF THE INSTITUTION

This is to certify that **Dr.NISHAN .A,** Post Graduate student (2014–2017) in the Department of Conservative Dentistry and Endodontics, K.S.R. Institute of Dental Science and Research, has done this dissertation titled**"EVALUATION OF ANTI E-FAECALIS EFFICACY AND PENETRATION DEPTH OF CURCUMIN LONGA MODIFIED SEALER-AN IN VITRO CLSM STUDY"**under our guidance and supervision in partial fulfillment of the regulations laid down by **TheTamil Nadu Dr. M.G.R. Medical University**, Chennai – 600 032 for **M.D.S.,** (Branch – IV) **CONSERVATIVE DENTISTRY AND ENDODONTICS** degree examination.

Seal & Signature of H.O.D.

Dr.K. SIVAKUMAR.,M.D.S

PROFESSOR

Seal & Signature of Principal

Dr.G.S.KUMAR.,M.D.S

PRINCIPAL

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation titled**"EVALUATION OF ANTI E-FAECALIS EFFICACY AND PENETRATION DEPTH OF CURCUMIN LONGA MODIFIED SEALER-AN IN VITRO CLSM STUDY"**is a bonafide research work done by **Dr.NISHAN.A** in partial fulfillment of the requirements for the degree of **MASTER OF DENTAL SURGERY** in the speciality of **CONSERVATIVE DENTISTRY AND ENDODONTICS**

Date:

Place: Signature of the Guide

DR.SIVA KUMAR.M.D.S

PROFESSOR&HOD

K.S.R. INSTITUTE OF DENTAL SCIENCE AND RESEARCH

TIRUCHENGODE

DECLARATION BY THE CANDIDATE

TITLE OF DISSERTATION	**EVALUATION OF ANTI E-FAECALIS EFFICACY AND PENETRATION DEPTH OF CURCUMIN LONGA MODIFIED SEALER-AN IN VITRO CLSM STUDY**
PLACE OF STUDY	K.S.R. Institute of Dental Science and Research
DURATION OF THE COURSE	3 Years
NAME OF THE GUIDE	Dr. Sivakumar.K
HEAD OF THE DEPARTMENT	Dr. Sivakumar.K

I hereby declare that no part of the dissertation will be utilized for gaining financial assistance for research or other promotions without obtaining prior permission of the Principal, K.S.R. Institute of Dental Science and Research, Tiruchengode. In addition, I declare that no part of this work will be published either in print or in electronic media without the guide who has been actively involved in dissertation. The author has the right to publish this study solely with the prior permission of the Principal, K.S.R. Institute of Dental Science and Research, Tiruchengode.

Head of the Department **Signature of the candidate**

Acknowledgement

I express my sincere thanks to Chairman **Thiru. Lion. Dr. K.S. Rangasamy,MJF.,**Principal **Dr. G.S. Kumar, M.D.S.,**the Head of the Department of Conservative Dentistry and Endodontics, **Dr. Sivakumar Kailasam, M.D.S.,** KSR Institute of Dental Science and Research, Thiruchengode, for permitting me to pursue this course and avail the facilities of this college.

With gratitude I thank my Professor **Dr. Siva Kumar, M.D.S.,** my guide, for his support and valuable guidance through the journey of my course and main dissertation.

My sincere thanks to **Dr. K. Karthick, M.D.S.,** Reader, Department of Conservative Dentistry and Endodontics, for his constant guidance and immense support with utmost patience during M.D.S. course and main dissertation.

I would also like to engrave here, my heartfelt respects and thanks to all my teachers in the department,**Dr.Sebeena Mathew,Dr. Harikaran M.D.S.,Dr. Deepa, M.D.S., Dr.Boopathi M.D.S.** and other staff members for their constant encouragement in all aspects of my career as an MDS student.

I would also like to thank my father **M.Abul Fazil ,**my mother **M.Dilshad** my brothers **Jazeel** and **Juhan** whose expeditious encouragement and prayers have always been a pillar of support for me.

I extend my heartfelt thanks to my colleagues **Dr.Sreedev.C.P** and **Dr.Iswarya.R.Raju** for their help, advice and support.

I thank the librarian **Mr. R. Madeshwaran, M.A., B.Ed., M.Phil.,** for his valuable work in helping me to access and collect the articles and text books for this dissertation.

I like to thank **Dr. Malini** ,V Clin bio,Sri Ramachandra University for her ideas and guidance during confocal laser scanning microscope imaging.

I like to thank **Dr.Prathaban Munisamy,**Department of biotechnology,KSR Arts and Science College for his ideas and guidance in inoculation and culturing of E.faecalis.

I extend my thanks to **Spy Printers**, Erode, for their help in compiling the dissertation, printing and binding.

Last but not the least; I thank **God, the Almighty**, for blessing me abundantly and for giving me the time,wisdom and inclination to write this main Dissertation.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF BAR DIAGRAMS

INTRODUCTION

Microorganisms are primary etiologic agents in pulpal and periapical diseases [1,2]. The purpose of endodontic therapy is to eliminate bacteria and their by-products from the infected root canal system and prevent subsequent reinfection[3]. Chemomechanical preparation (instrumentation and irrigation) and intracanal medicaments significantly reduce microorganisms inside the infected root canal. However, it is virtually impossible to completely eliminate all microbes from root canal system in every case [4]. Hence the use of endodontic obturation materials and sealers with antimicrobial activity is considered beneficial in further reducing the concentration of residual microorganisms[4,5]. Root canal sealers with antimicrobial activity can help to improve the success rate of endodontic treatment and are especially advantageous in clinical situations where there is persistent or recurrent infection [6] . The persistence of microorganisms in dentinal tubules, lateral canals and apical ramifications after root canal treatment has been reported [7-9]. If the filling provides a good seal, it will only impair the exit of bacteria entrapped in the root canal system. However, to eradicate the remaining microorganisms, the antimicrobial activity of the sealer could play an important role .[10-11]

The need for a biocompatible material that induces the formation of mineralized tissue and also has suitable flow rate and manipulation, led to the development of MTA-based root canal sealers.[12]

MTA Sealer did not show significant inhibition against E.faecalis despite their high pH.[13,14] So the anti microbial efficacy of MTA based sealers was questionable and thus a modification was done by adding curcumin longa(turmeric) extract.

The curcumin longa (turmeric) is extensively used as a spice, food preservative and coloring material in India, China, South East Asia. It has been used in traditional medicine for the treatment of numerous diseases. Curcumin(diferuloylmethane), the main yellow bioactive component of turmeric has been shown to have a wide spectrum of biological actions including anti microbial, anti inflammatory and anti oxidant activities.[15-19] Various studies have shown the antimicrobial effects of extracts of roots of curcumin longa on various micro organisms.[20-23]

Enterococcus faecalis is often used in research that aims to evaluate the antimicrobial properties of endodontic materials. It seems to play a significant role in the etiology of persistent periradicular lesions [24]. Enterococcus faecalis possesses several virulence factors that contribute to its ability to survive the effects of conventional root canal therapy[25]. Besides, this Gram-positive facultative anaerobe is able to invade dentine tubules and bind to collagen [26].

To achieve the goal of thorough canal obturation not only must the tissue debris and contaminates be removed, but also the filling materials and techniques used to place them must achieve a high level of adaptability to the cleaned root canal space and dentin walls, including penetration into the dentinal tubules if possible.[27]

Many different techniques have been used to measure the effectiveness of treatment procedures against bacteria in dentinal tubules[28-31]. One of the major challenges with the traditional dentin block model has been the difficulty to obtain a strong, deep, and comparable infection in dentin. The lack of heavy presence of bacteria in dentin canals makes it difficult to measure the comparative effectiveness of various disinfecting agents. Therefore, a dentin infection model was developed to secure a predictable, dense and deep penetration of bacteria in dentin canals using centrifugation[31,32]. Results

obtained using this model and viability staining with Confocal Laser Scanning Microscopy (CLSM) have shown reproducible data on dentin disinfection in different conditions, including different biofilm age [33], length of disinfectant exposure [34], and combinations of disinfecting agents .[35]

The aim of this study was to evaluate the antimicrobial efficacy of curcumin modified bioceramic sealer at 3 time intervals (7,21,45 days) along with zinc oxide eugenol sealer and depth of penetration.

AIM

To evaluate the anti E.faecalis efficacy and depth of penetration of curcumin modified sealer using Confocal Laser Scanning Microscope(CLSM).

OBJECTIVES

The main objective is to

- Evaluate the anti E.faecalis efficacy of curcumin modified sealer(MTAC) and zinc oxide eugenol(ZOE).
- Evaluate the depth of penetration into dentinal tubules by curcumin modified sealer and zinc oxide eugenol.

REVIEW OF LITERATURE

Ørstavik in 1996 recommended the use of endodontic sealer with antibacterial properties to decrease or avoid future growth of the remaining microorganisms[6].

Spanberg in 1973 stated that to eradicate the remaining microorganism in the root canal ,the anti microbial activity of sealer could play an important role[10].

Peters in 2001 found out that even after chemo mechanical preparation bacterias were left inside root canal. Depending on the host– parasite equilibrium and the nutrition available after root filling, these bacteria may be of importance in recalcitrant apical periodontitis[7].

P.N.R Nair in 2005 reported persistence of micro organisms in dentinal tubules, lateral canals and apical ramifications after root canal treatment[8]. This study was an in vivo evaluation of intracanal microbial status of apical root canal system of mesial roots of human mandibular first molars with primary apical periodontitis immediately after one-visit endodontic treatment. The residual intracanal infection was confirmed by correlative light and transmission electron microscopy. Sixteen diseased mesial roots of mandibular first molars were treated endodontically, each in one visit. Mesio-buccal canals were instrumented using stainless steel hand files and mesio-lingual canals with a nickel-titanium rotary system. The canals were irrigated with 5.25% sodium hypochlorite (NaOCl) during the instrumentation procedures, rinsed with 10 mL of 17% ethylenediamine tetraacetic acid (EDTA), and obturated with gutta-percha and zinc oxide eugenol cement. Thereafter, the apical portion of the root of each tooth was removed by flap-surgery. The specimens were fixed, decalcified, subdivided in horizontal plane, embedded in plastic, processed, and evaluated by correlative light and transmission electron microscopy. Fourteen of the 16 endodontically treated teeth revealed residual

intracanal infection after instrumentation, antimicrobial irrigation, and obturation. The microbes were located in inaccessible recesses and diverticula of instrumented main canals, the intercanal isthmus, and accessory canals, mostly as biofilms.

Love in 2001 postulated that a virulence factor of E. faecalis in failed endodontically treated teeth may be related to the ability of E.faecalis cells to maintain the capability to invade dentinal tubules and adhere to collagen in the presence of human serum[26].The aim of this study was to identify a possible mechanism that would explain how E.faecalis could survive and grow within dentinal tubules and reinfect an obturated root canal. Cells of Streptococcus gordonii DL1,Streptococcus mutans NG8 ,or E.faecalis JH2-2 were grown in brain heart infusion broth containing various amounts of human serum for 56 days. The ability of three species to invade dentin and bind to immobilized type 1 collagen in the presence of human serum was assessed by dentine invasion and microtitre well experiments. All three species remained viable over the period of the experiment when grown in human serum. Cells of all three bacteria were able to invade dentine and bind to immobalized collagen. Human serum inhibited dentine invasion and collagen adhesion by S.gordonii DL1 and S.mutans NG8 whilst dentine invasion by E.faecalis JH2-2 was reduced in the presence of serum , but not inhibited , and binding to collagen was enhanced.

Stuart in 2006 stated that the prevalence of E. faecalis is low in primary endodontic infections and high in persistent infections. E. faecalis is also more commonly associated with asymptomatic cases than with symptomatic ones. Although E. faecalis possesses several virulence factors, its ability to cause periradicular disease stems from its ability to survive the effects of root canal treatment and persist as a pathogen in the root canals and dentinal tubules of teeth[36].

Gomes in 2006 found out that E.faecalis was detected as frequently in teeth with necrotic pulp as in teeth with failing endodontic treatment when a Polymerize Chain Reaction PCR analysis was used[37]. The objective of this study was to investigate the presence of *Enterococcus faecalis* in endodontic infections by culture and polymerase chain reaction analyses. Microbial samples were obtained from 50 teeth with untreated necrotic pulps (primary infection) and from 50 teeth with failing endodontic treatment (secondary infection). Culture techniques were used including serial dilution, plating, incubation, and biochemical identification. For PCR detection, samples were analyzed using a species-specific primer of the 16S rDNA and the downstream intergenic spacer region. Culture and PCR detected the test species in 23 of 100 and 79 of 100 of the teeth, respectively. *E faecalis* was cultured from 2 (4%) of 50 necrotic canals and from 21 (42%) of 50 root-treated canals. PCR detection identified the target species in 41 (82%) and 38 (76%) of 50 primary and secondary infections respectively.

Kayaoglu and Orstavik in 2004 stated that the most-cited virulence factors are aggregation substance, surface adhesins, sex pheromones, lipoteichoic acid, extracellular superoxide production, the lytic enzymes such as gelatinase and hyaluronidase, and the toxin, cytolysin. Each of them may be associated with various stages of an endodontic infection as well as with periapical inflammation[38] .

Dipthi Rai in 2008 stated that the curcumin inhibits bacterial cell division, by perturbing the cytokinetic Z-ring through a direct interaction with FtsZ. These findings may help to design potent curcumin analogues with improved stability and bioavailability. FtsZ, a prokaryotic homologue of eukaryotic cytoskeletal protein tubulin, polymerizes to form a Z-ring at the mid cell that orchestrates bacterial cell division .FtsZ is shown to be essential for bacterial cell division and viability . Previous studies have

shown that the perturbation of FtsZ functions by natural compounds and chemical agents leads to inhibition of bacterial proliferation[39].

Prasanna Neelakantan in 2013 found out that Sodium hypochlorite (3%) showed maximum antibacterial activity against E.faecalis biofilm formed on the tooth substrate, followed by curcumin and CHX(chlorhexidine). Considering the potential for undesirable properties of NaOCl, the use of herbal alternatives in endodontics might prove to be advantageous[40] . To evaluate the antimicrobial efficacy of curcumin against Enterococcus faecalis biofilm formed on tooth substrate in vitro. Sodium hypochlorite (NaOCl) and chlorhexidine (CHX) served as standards for comparison. Biofilms of E.faecalis were formed on instrumented, extracted human teeth (n = 96). At the end of the 2nd day, 2nd week and 8th week, specimens were treated for 30 min with one of the test solutions or saline (control) and the surviving colony-forming units (CFU/mL) was recorded. Results were analyzed by Kruskal-Wallis test and Dunnet test for pair-wise comparison with Bonferroni correction ($p = 0.05$). Only NaOCl showed complete eradication of bacteria at all time periods. In the 2-day and 2nd week biofilms, curcumin and NaOCl showed complete inhibition, which was significantly lower than the CFU recovered in the CHX and saline groups ($p < 0.05$). In 8 week biofilms, samples treated with curcumin showed 553 ± 137.6 CFU/mL, which was significantly higher than NaOCl (0 CFU/mL), but significantly lower than CHX (2551 ± 129.8) and saline control ($1.42 \times 1011 \pm 2.12 \times 1010$; $p < 0.05$).

Mandroli in 2013 stated that curcumin has the potential to be developed into medicament for the treatment of various endodontic diseases[41]. Aim of the study was to investigate the anti-bacterial potential of curcumin, against standard strains of common endodontic bacteria. The bacterial strains of Streptococcus mutans (ATCC 35668), Actinomyces viscosus (ATCC 10048), Lactobacillus casei (ATCC 334), Porphyromonas

gingivalis (ATCC 33277), Prevotella intermedia (ATCC 25611), Enterococcus faecalis (ATCC 29212) from the stock were revived by plating on blood agar medium. Isolated colonies were transferred to sterile Brain Heart Infusion (BHI) broth and once again incubated overnight. The growth concentration was adjusted to 5 X 105 organisms / ml by using 0.5 McFarland's turbidity standard. MIC was determined, by serial broth dilution of curcumin to 500, 250,125, 62.5, 31.25, 16, 8, 4, 2, 1 µg /ml. respectively. The tubes were then incubated for 24 hours at 37^0C. The last tube with clear supernatant was considered to be without any growth and taken as MIC value. Mean MIC values of curcumin were as follows: S. mutans (333.33 µg /ml), A. viscosus (167.67 µg /ml), L. casei (125 µg /ml), P. gingivalis (125 µg /ml), and P. intermedia (208.33 µg /ml). There was no action against E. faecalis.

Mithra Hegde in 2012 found out that the extracts of Curcuma longa showed antimicrobial activity against the tested organisms[63] .

Mishra in 2005 stated that curcumin glycine bioconjugates has anti bacterial and anti fungal properties[17].

Rambir in 2002 suggested that essential oil fraction from turmeric possesses significant antibacterial activity at very low concentration on pathogenic gram positive S. aureus bacteria[20]. Curcuma longa rhizome extracts were evaluated for antibacterial activity against pathogenic strains of Gram-positive (Staphylococcus aureus, Staphylococcus epidermidis) and Gram-negative (Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium) bacteria. Essential oil was found to be most active and its activity was compared to standard antibiotics gentamycin, ampicillin, doxycycline and erythromycin in these strains. Only the clinical isolate of S. aureus showed more sensitivity towards essential oil fraction than the standard strain. The use of essential oil

from turmeric as a potential antiseptic in prevention and treatment of antibacterial infections has been suggested.

Orstavik in 2005 stated that root canal sealer is essential to not only assist in filling but should also penetrate into small inaccessible areas such as dentinal tubules[42].

Tanomaru Filho M in 2007 stated that the ability to penetrate into the dentinal tubules may be beneficial to control or kill bacteria that are located there[43].

Suresh Chandra in 2012 stated that the advantages of a deeper sealer penetration are their potential antibacterial effects and entombing the viable bacteria within tubules by isolating them from potential nutrient sources. The potential for bacteria to colonize dentinal tubules has been well established[44]. The aim of this in vitro study was to evaluate the depth of penetration of 4 different endodontic resin sealers into the radicular dentinal tubules with the aid of confocal microscopy. Methods: Eighty single-rooted teeth were instrumented and divided into 4 groups composed of 20 teeth each. The samples were obturated with AH Plus, RealSeal, EndoRez, and RoekoSeal resin sealers respectively. The core material in all the groups was Resilon. The teeth were sectioned at the coronal, middle and apical thirds and viewed under confocal microscope to determine the depth of penetration of the sealer into the dentinal tubules. Results: The results showed that the maximum penetration was exhibited by RealSeal resin sealer, followed by AH Plus, RoekoSeal, and EndoRez. The coronal third showed the maximum penetration, followed by middle third and least at the apical third.

Torabinejad in 1995 using the original Loma Linda formula, found that MTA was effective against some facultative microorganisms but not against other bacterial strains, including Enterococcus faecalis[45.] In addition to having good sealing ability, root end filling materials should ideally have some antibacterial activity to prevent bacterial

growth. This investigation compared the antibacterial effects of amalgam, zinc oxide-eugenol, Super EBA and a mineral trioxide aggregate on nine facultative bacteria such as Streptococcus faecalis, Streptococcus mitis, Streptococcus mutans, Streptococcus salivarius, Lactobacillus species, Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis, and Escherichia coli B and seven strict anaerobic bacteria, Prevotella (Bacteroides) buccae, Bacteroides fragilis, Prevotella (Bacteroides) intermedia, Prevotella (Bacteroides) melaninogenica, Fusobacterium necrophorum, Fusobacterium nucleatum, and Peptostreptococcus anaerobius. After growing these bacteria on solid media, freshly mixed and 24-h set test materials were placed on the surface of these inoculated media and incubated in the appropriate atmosphere for 24 to 48 h at 37 ^{0}C. Impregnated discs with the Super EBA liquid were used as positive controls. The antibacterial effects of each material were measured in millimeters and the data were analyzed using one-way and two-way analysis of variance and Scheffé tests to determine the statistical differences between the antibacterial effects of the test materials. Impregnated discs with Super EBA liquid caused varying degrees of growth inhibition for both facultative and strict anaerobic bacteria. Both types of amalgam had no antibacterial effect against any of the bacteria tested in this study. Mineral trioxide aggregate had an antibacterial effect on some of the facultative bacteria and no effect on any of the strict anaerobic bacteria. Zinc oxide-eugenol and Super EBA pastes had some antibacterial effects on both types of bacteria tested.

I.M.Saleh in 2004 and **Zhang** in 2007 stated that MTA based sealers doesn't have anti bacterial properties against E.faecalis[46,47] .

Al –Hezaimi in 2009 stated that the origin of MTA as well as the type of preparation may affect its antimicrobial characteristics[14]. The antimicrobial effects of 4 mineral trioxide aggregate (MTA) preparations, 2 white-colored (WMTA-1, WMTA-2)

and 2 gray-colored (GMTA-1, GMTA-2), against C. albicans and E. faecalis were assessed in vitro. Minimal inhibitory concentration (MIC) for each preparation was determined using the tube dilution test and Sabouraud agar media for C. albicans and brain heart infusion media for E. faecalis. Broth tubes were prepared and divided into experimental and control groups. Aliquots of each of the tested microorganisms were taken from a stock culture and added to each experimental and positive control group. All groups were incubated at 37°C and evaluated for turbidity at 24-hrs, 48-hrs, and 72-hour time periods. Samples of 0.1 mL from each of the experimental and control tubes were subcultured on agar or brain heart infusion plates to confirm visible signs of bacterial or fungal growth. MIC of MTA against the 2 microorganisms tested varied among the 4 preparations tested. WMTA-1 and WMTA-2 inhibited C. albicans growth at concentrations of 3.125 mg/10 mL and 25 mg/10 mL, respectively, and statistically significant differences were found between WMTA-1 and WMTA-2 (P<.001). WMTA-1 and WMTA-2 inhibited E. faecalis growth at concentrations of 12.5 mg/10 mL and 50 mg/10 mL, respectively, and statistically significant differences were found between WMTA-1 and WMTA-2 (P<.001). GMTA-1 and GMTA-2 inhibited E. faecalis growth at concentrations of 12.5 mg/10 mL and 3.125 mg/10 mL, respectively, and statistically significant differences were found between GMTA-1 and GMTA-2 (P<.001). Both GMTA-1 and GMTA-2 inhibited C. albicans growth at a concentration of 3.125 mg/10 mL and no statistical differences were found between the preparations. Subculture of the broth tubes in agar or brain heart infusion plates confirmed the turbidity test result.

Estrela in 2000 stated that MTA sealer did not show effective inhibition against E. faecalis[13].

Miyagak in 2006 reported no antimicrobial activity of MTA against E.faecalis in agar diffusion test[48]. The purpose of this study is to evaluate the antimicrobial activity of

the endodontic sealers: N-Rickert, Sealapex, AH Plus, Mineral Trioxide Aggregate (MTA) and portland cement. The Agar diffusion method was used in plates previously inoculated with the following microorganisms: C. albicans, S. aureus, E. faecalis, E. coli. The diameters of microbial inhibition zones were measured after 24 hours of incubation in kiln at 37°C. According to the methodology used, it was possible to conclude that only the sealers AH Plus and N-Rickert presented antimicrobial activity against C. albicans, S. aureus, and E. coli; no antimicrobial activity in MTA, Sealapex and portland cement was observed. N-Rickert presented the largest inhibition zones varying from 8 to 18 mm and the microorganism E. faecalis was resistant against all sealers tested.

Sipert in 2005 reported that MTA demonstrated antimicrobial activity against E. faecalis[49] .

McHugh in 2004[50], **Al-Hezaimi** in 2006[51] and **Holt** in 2007 [52] reported that grey MTA showed greater E. faecalis growth inhibition than white MTA.

ARMAMENTARIUM USED

1. Extracted single canal tooth
2. 3% thymol solution
3. Diamond disc and mandrel
4. Airotor handpiece
5. Micromotor hand piece(NSK)
6. K files
7. Saline
8. 5.25 % sodium hypochlorite solution
9. Disposable syringe
10. X smart (dentsply maillefer)
11. Protaper rotary files
12. Protaper gutta-percha F3 size
13. MTA plus
14. Scaler unit(satelec)
15. E.faecalis(ATCC 29212)
16. Rhodamine B dye
17. Laminar flow chamber
18. Hydro alcoholic curcumin extract
19. Zinc Oxide Eugenol Sealer

SOURCE OF DATA

Extracted single canal tooth has been collected from Department of Oral and Maxillofacial Surgery in KSR Institute of Dental Science and Research. Study was conducted in Department of Conservative Dentistry and Endodontics, KSR Institute of Dental Science and Research. Bacterial inoculation and biofilm development was done in Department of Biotechnology,KSR Arts and Science College. Confocal Laser Scanning Microscopic imaging has been done in Vclin bio research centre, Sri Ramachandra University,Chennai.

INCLUSION CRITERIA

- Uniradicular teeth
- Teeth with single root canal

EXCLUSION CRITERIA

- Multi radicular teeth
- Teeth with multiple canals
- Teeth with ribbon shaped canals.
- Teeth with any anomalies.
- Teeth with root resorption.

MATERIALS AND METHODS

Anti Bacterial Efficacy

Dentin Specimen Preparation

45 single rooted teeth were collected from the Department of oral surgery ,KSR Institute of Dental Science and Research. According to a previously described protocol 90 semi cylindrical halves were fractured and shaped into 4x4x2 mm in size. The cementum layer on the external dentin surface was removed so that the thickness of samples remains same. The smear layer on both sides of the specimen was removed by immersion in 5% NaOCl and 6% citric acid each for four minutes in an ultrasonic bath. The prepared dentin specimens with their canals side up were placed inside filter tube.

Dentin Infection of E.Faecalis

E.faecalis ATCC 29212 was used as the test organism and grown in air at 37^0C overnight on BHI(brain heart infusion) agar plates. The bacteria were harvested and suspended in BHI broth. Cell density was spectrophotometrically standardized into 3×10^6colony forming unit/ml.

Following a protocol described in detail by Ma et al 500 micro litre E.faecalis suspension in BHI broth was added to each filter tube with the dentin specimen inside. The tubes were centrifuged at 1400 g, 2000g , 3600 g and 5000 g in a sequence twice each for 5 minutes. A fresh solution of bacteria was added between every centrifugation. All tubes were then incubated for 3 weeks under the same conditions to allow biofilm growth and maturation in the dentinal tubules. Fresh BHI broth was changed once a week.

The dentin specimens were taken out of each tube rinsed in sterile water for 1 minute and air dried. The outer surface of the specimens were sealed with nail varnish. The 90 infected dentin halves with 3 week old E.faecalis biofilms were randomly allocated to 2 major groups. Group 1(n=45) is zinc oxide eugenol(ZOE) and group 2(n=45) is curcumin modified MTA sealer(MTAC).

Sealer placement

Sealer was placed on the dentin surface of root canal wall. All dentin samples were placed in 100% humidity for 7,21,45 days(n=15) after which the sealer was scraped off.

CLSM Examination

Two semi cylindrical dentin halves of each group were examined by viability staining and CLSM. After scraping of sealers the dentin halves were rinsed in sterile water and vertically fractured through the root canal into 2 halves to expose a fresh surface of longitudinally fractured dentinal tubules for CLSM examination as previously described [32] (fig.1).

LIVE/DEAD backlight bacterial viability staining containing Fluorescein diacetate(FDA) and Propidium Iodide(PI) was used to stain a total of 180 fractured dentin specimens according to manufactures instructions. A confocal laser scanning microscope was used to view the fluorescence from the stained bacterial cells using 20 x lens. Four areas on the border of the root canal were randomly chosen on each of the 4 specimens in each group per time point for CLSM scanning. Images were acquired by EZ –C1 v.3.40 build 691 software at a 512×512 pixel scan area .

The confocal laser scanning microscope data were processed by imaris 7.2 software . The thresholds of the red and green fluorescence were manually set according to their respective fluorescence intensity and kept consistent for each sample. Live/dead ratio were automatically calculated by the software.The relative area percentage of red fluorescence to added (green and red) fluorescence indicated the proportion of killed cells. The relative area percentage after exposure to different sealers were subjected to mean and standard deviation and unpaired t test were used to isolate and compare the results at a significance level of $p<0.05$.

Depth of penetration

30 maxillary single rooted teeth stored in thymol solution were used in this study. The coronal portion was cut at the cementum level .Root canal preparation was done upto 0.5 mm short of working length up to protaper F3. The hand piece used was an electric engine at 250 rpm. Irrigation procedures were accomplished by using 2 ml of 5% sodium hypochlorite for each file used. To remove smear layer 3 ml of 6% citric acid was used. Finally the root canals were flushed with distilled water and canals dried with sterile paper points. F3 master cone was selected and sealers were mixed with rhodamine B dye and then obturated.

Group 1(n=15) zinc oxide eugenol(ZOE)

Group 2 (n=15) curcumin modified MTA sealer(MTAC)

After obturation, 2mm thick cross section from the apical section of the root was taken and analysed under CLSM.

CLSM Examination

The dentin segments were examined on a confocal laser scanning microscope .The respective absorption and emission wave lengths for the rhodamine B were 540 nm. Dentin samples were analyzed at 20 x for depth of penetration into tubules. The canal wall served as the starting point and sealer penetration into dentinal tubules were measured to a maximum depth of 1000 microns. Statistical significance for the mean depth of penetration of root canal sealers were determined using unpaired t test. The level of significance was set at $p<0.05$.

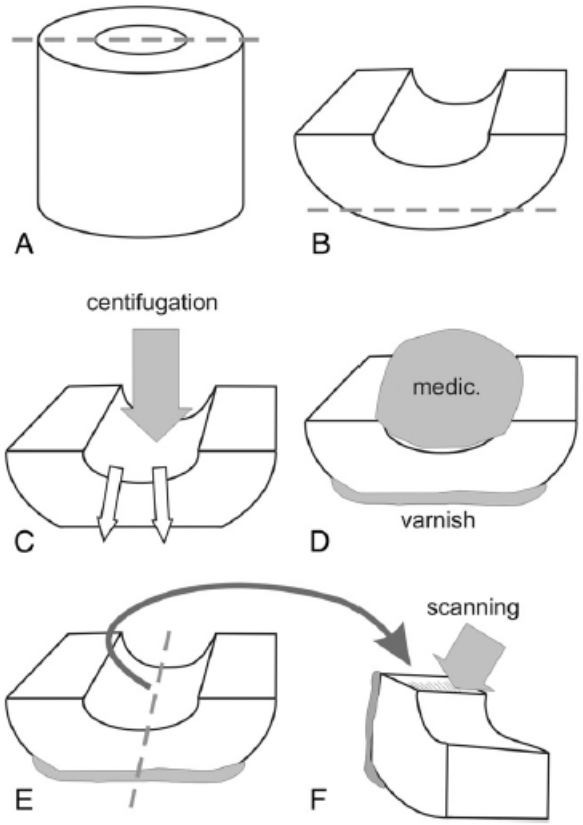


Figure 1: Schematic representation of specimen preparation for sealer placement and viewing under CLSM for evaluating anti bacterial efficacy

ARMAMENTARIUM

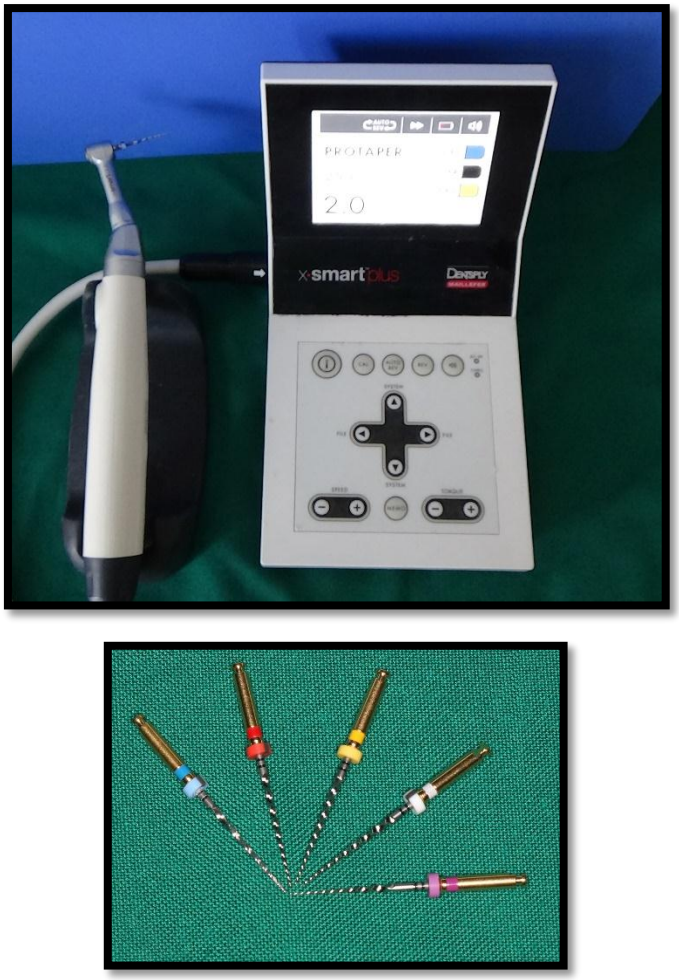


Figure 2 : Xsmart plus(Dentsply) and Protaper files S1,S2,F1,F2,F3

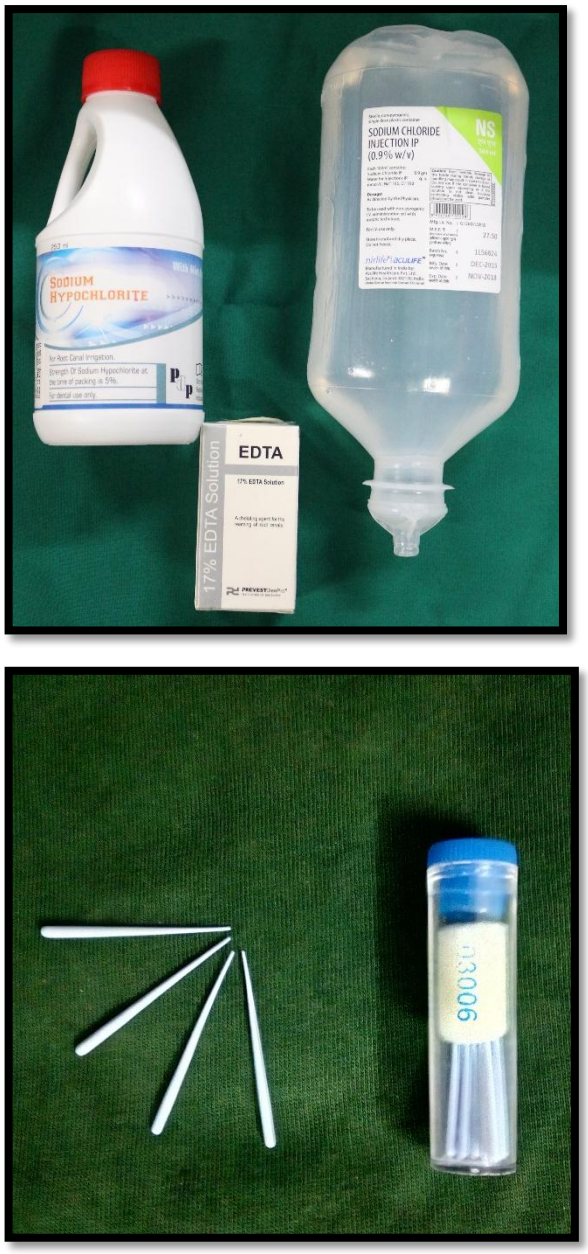


Figure 3 : Irrigant solutions and gutta-percha cones.

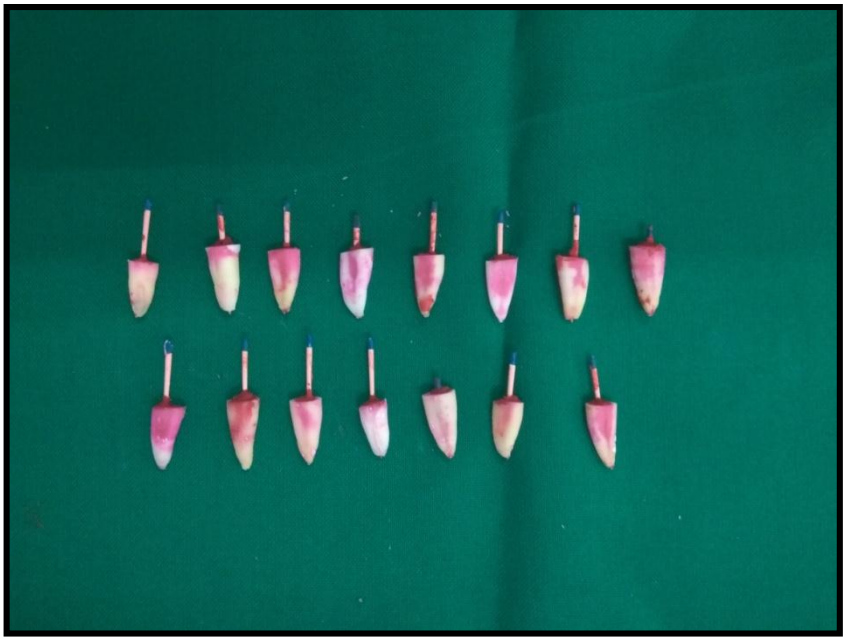


Figure 4: Teeth samples used in the study for analyzing depth of penetration

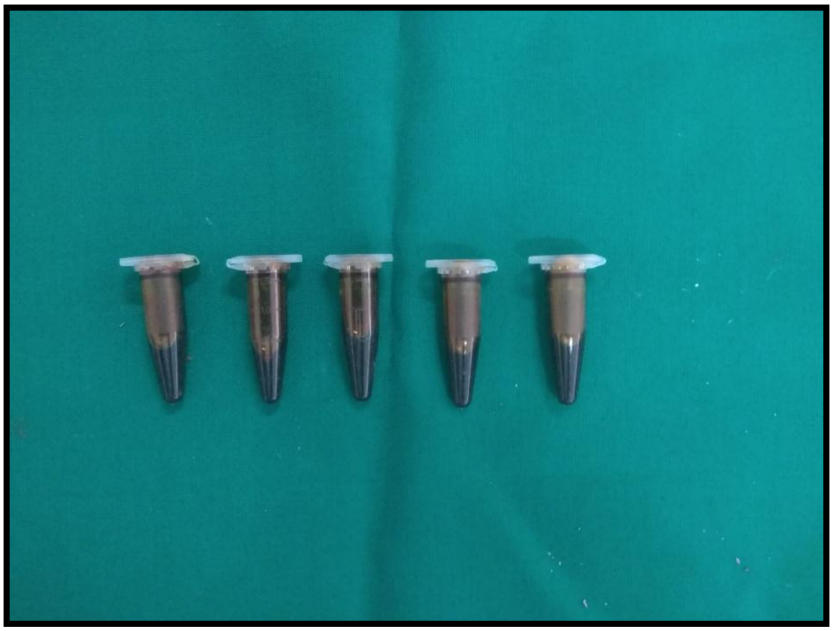


Figure 5: Hydro alcoholic extract of Curcumin

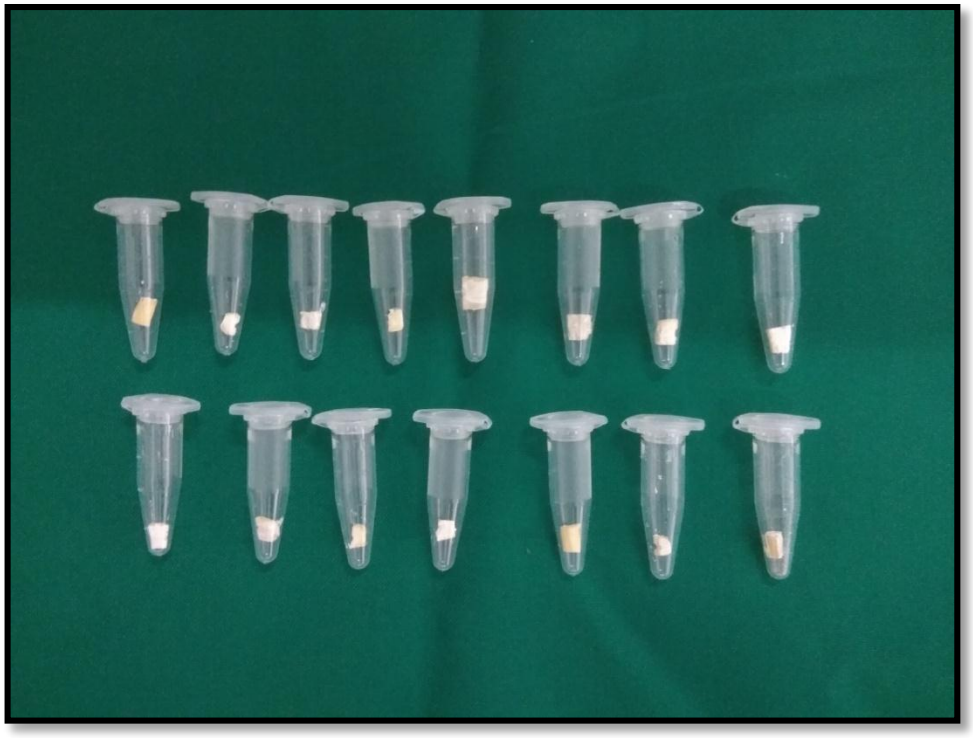


Figure 6: sample preparation after placement of zinc oxide eugenol sealer for evaluating anti bacterial efficacy

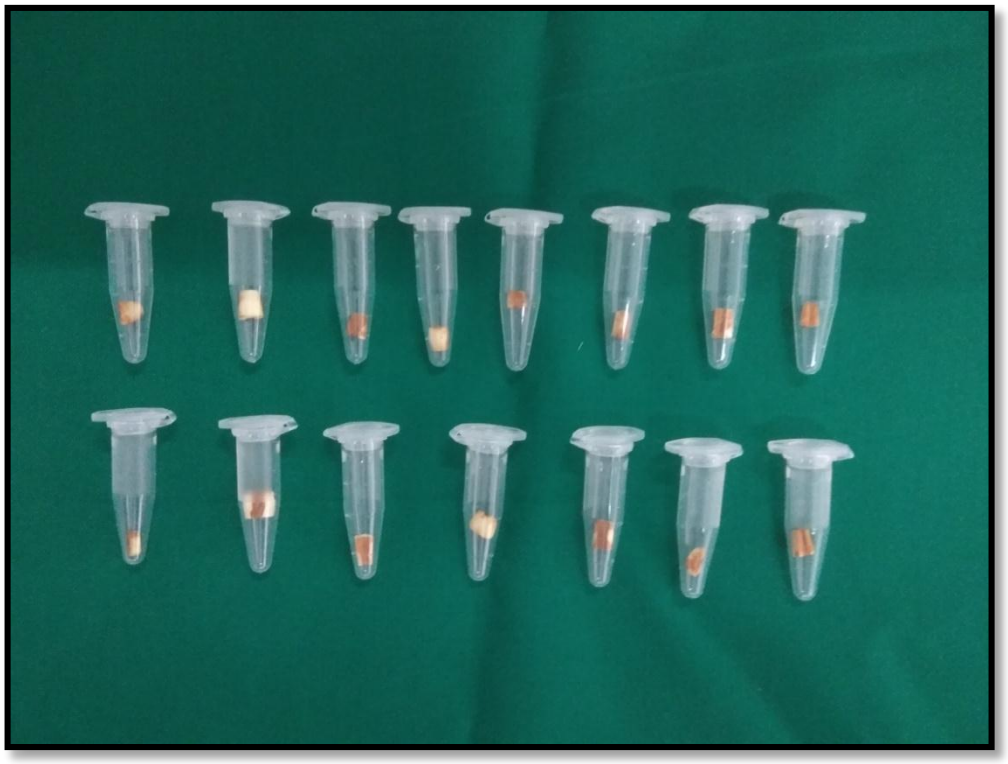


Figure 7:sample preparation after the placement of with curcumin modified MTA sealer for evaluating anti bacterial efficacy

Figure 8: MTA sealer used in the study

Figure 9 : Zinc oxide eugenol sealer used in the study.

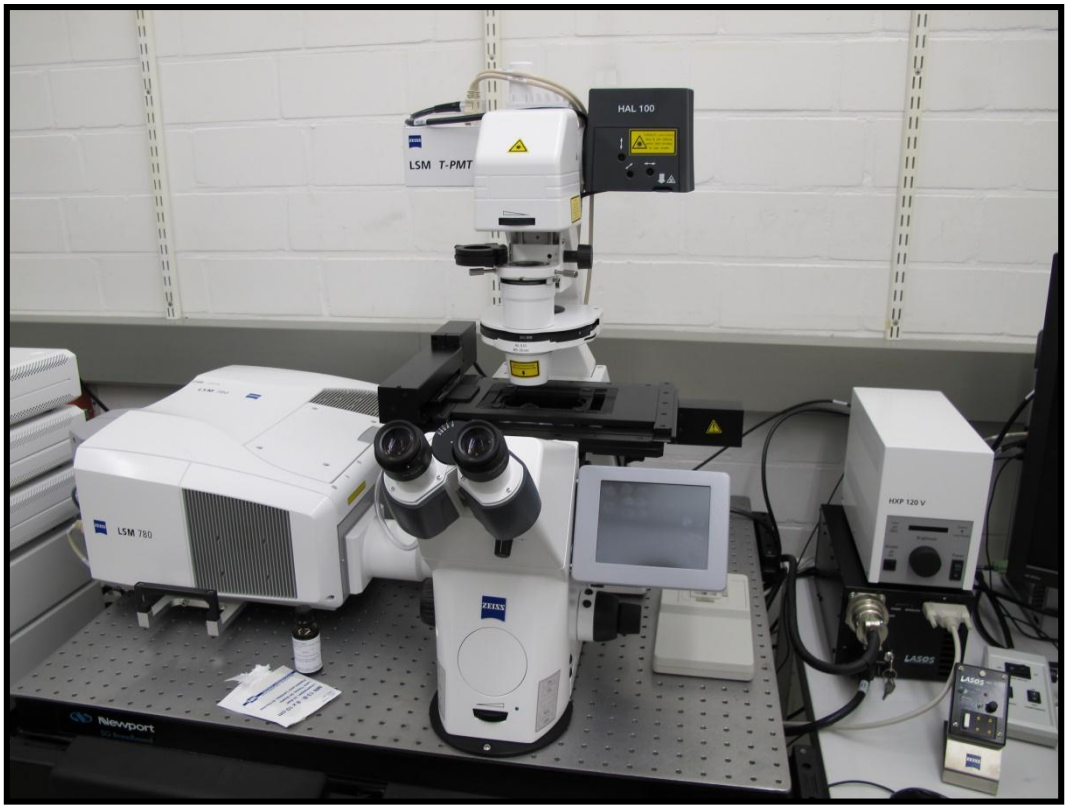


Figure 10: Confocal laser scanning microscope(CLSM)

RESULTS

sample	ZOE	MTAC
1	187.41	234.99
2	237.92	196.75
3	221.65	278.33
4	187.41	241.25
5	194.79	288.43
6	221.65	238.94
7	237.92	262.21
8	221.65	275.27
9	237.8	279.93
10	194.79	234.94
11	187.41	196.77
12	237.8	278.33
13	221.65	241.25
14	194.79	275.26
15	237.92	288.4

Table 1: Depth of penetration(μm) of zinc oxide eugenol sealer and curcumin modified MTA sealer into dentinal tubules

sample	7 days	21 days	45 days
1	3.6	1	0.6
2	1.8	0	0.7
3	7.8	0.1	2.3
4	3.6	18.2	1
5	37.1	2.8	9.8
6	5.2	1.1	10
7	3.4	0	9
8	2.1	0.2	2.9
9	2.4	5.4	6.4
10	2.6	0.6	0.8
11	1.8	9	3.7
12	7.8	0.8	0.7
13	5.2	1.1	1
14	3.6	0	2.3
15	5.2	0.7	2.9

Table 2:Relative area percentage of dead cell volume in dentinal tubules treated by curcumin modified MTA sealer

sample	7 days	21 days	45 days
1	18.1	8.2	2.9
2	31.6	3	0.1
3	48	5	0.1
4	15.9	7	0.6
5	8.2	0.3	0.9
6	7	5	1.6
7	0.6	18.1	1
8	8.5	31.6	1.9
9	9.4	48	1.2
10	12.6	0.8	1.6
11	8	0.1	0.3
12	17.4	5.5	0.8
13	15.2	15.9	0.1
14	2.9	9.3	0.8
15	0.1	0.8	0.6

Table 3: Relative area percentage of dead cell volume in dentinal tubules treated by zinc oxide eugenol sealer

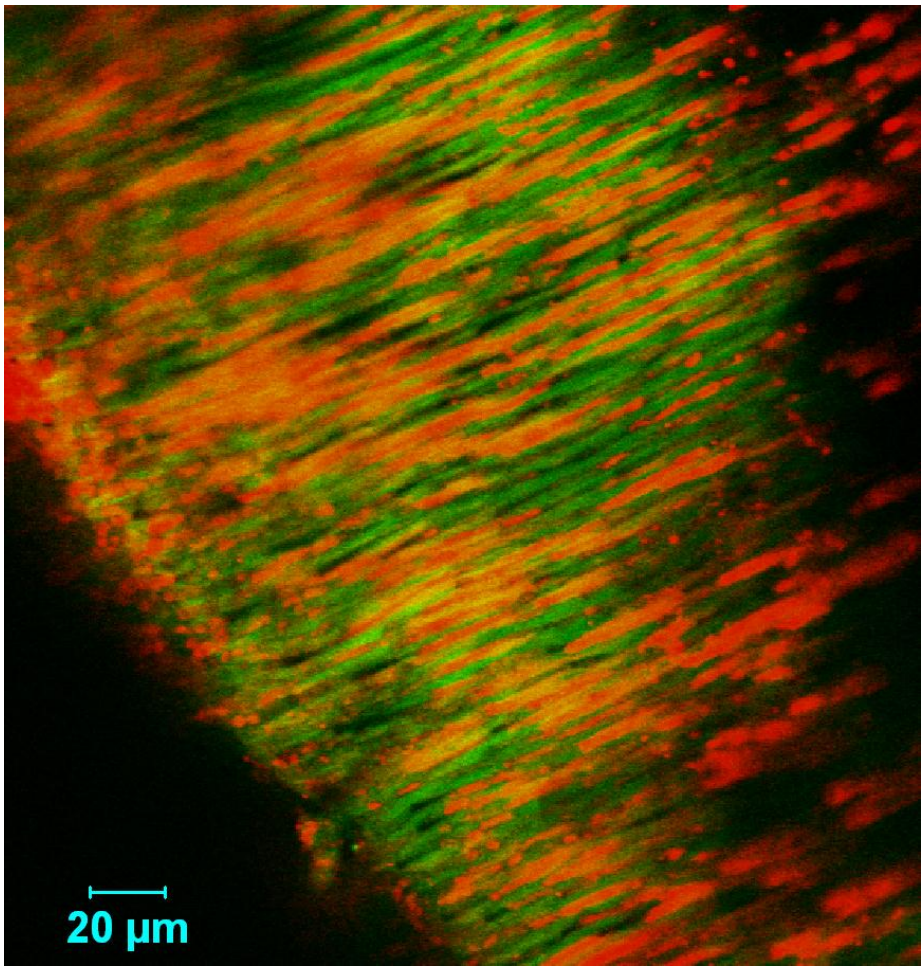


Figure 11: Anti E.faecalis efficacy of curcumin modified MTA sealer at 7 days

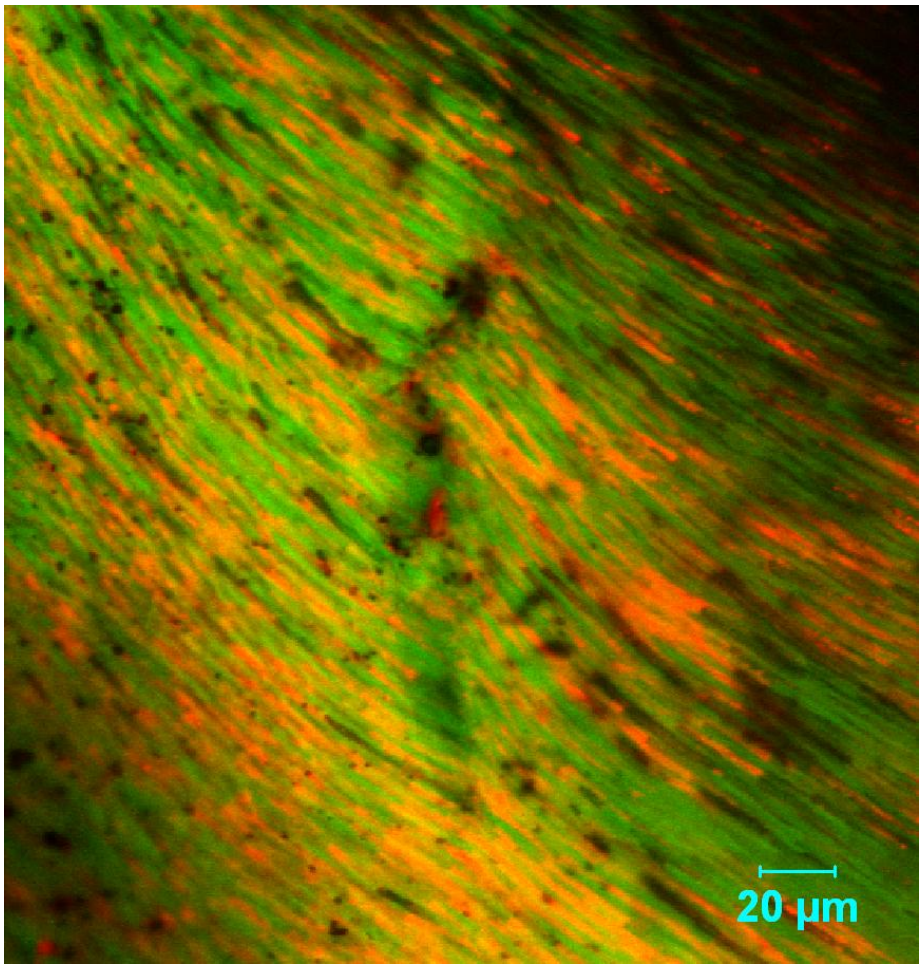


Figure 12: Anti E.faecalis efficacy of curcumin modified MTA sealer at 21 days

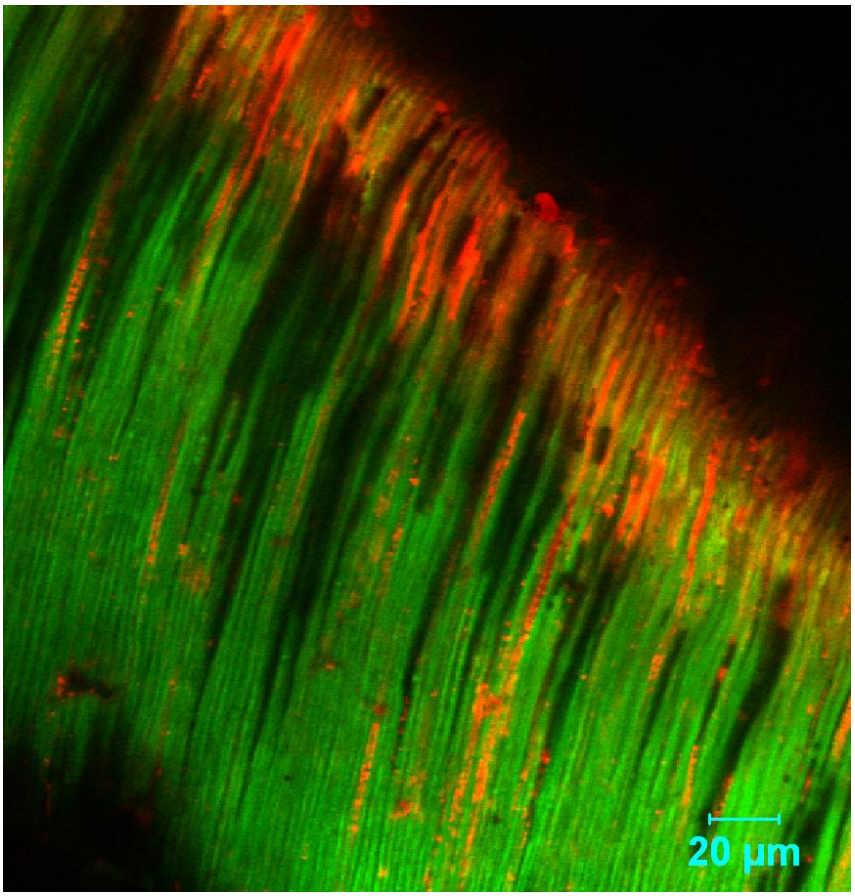


Figure 13 : Anti E.faecalis efficacy of curcumin modified MTA sealer at 45 days

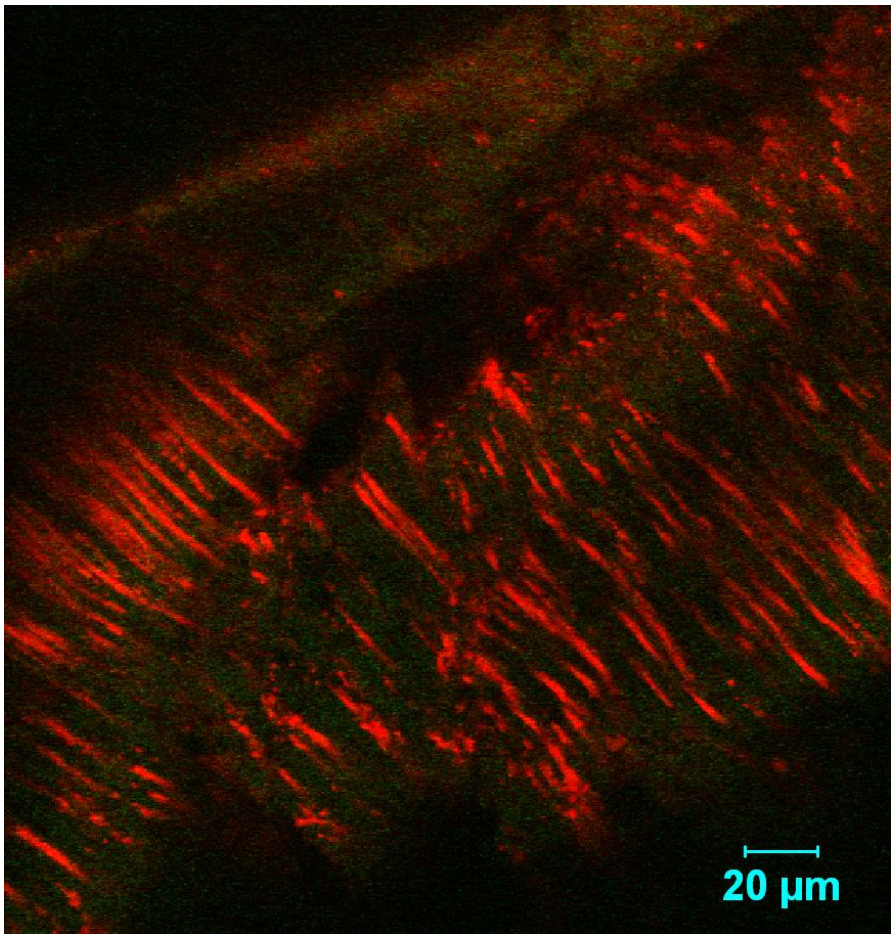


Figure 14: Anti E.faecalis efficacy of zinc oxide eugenol sealer at 7 days

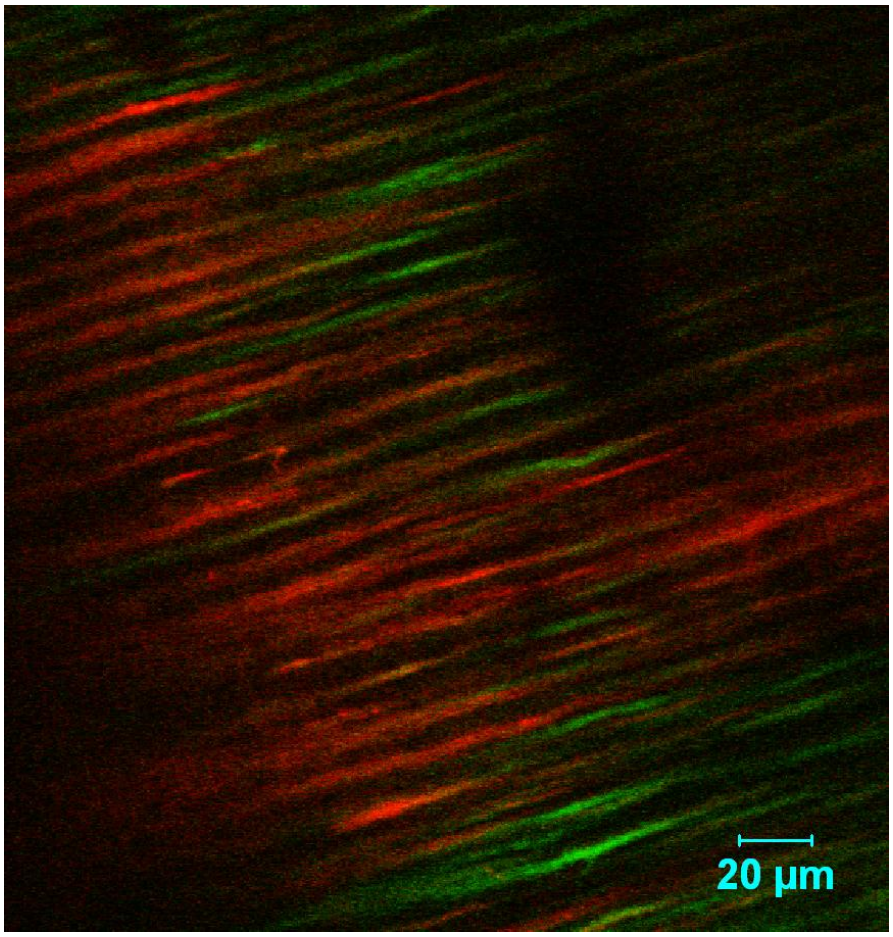


Figure 15: Anti E.faecalis efficacy of zinc oxide eugenol sealer at 21 days

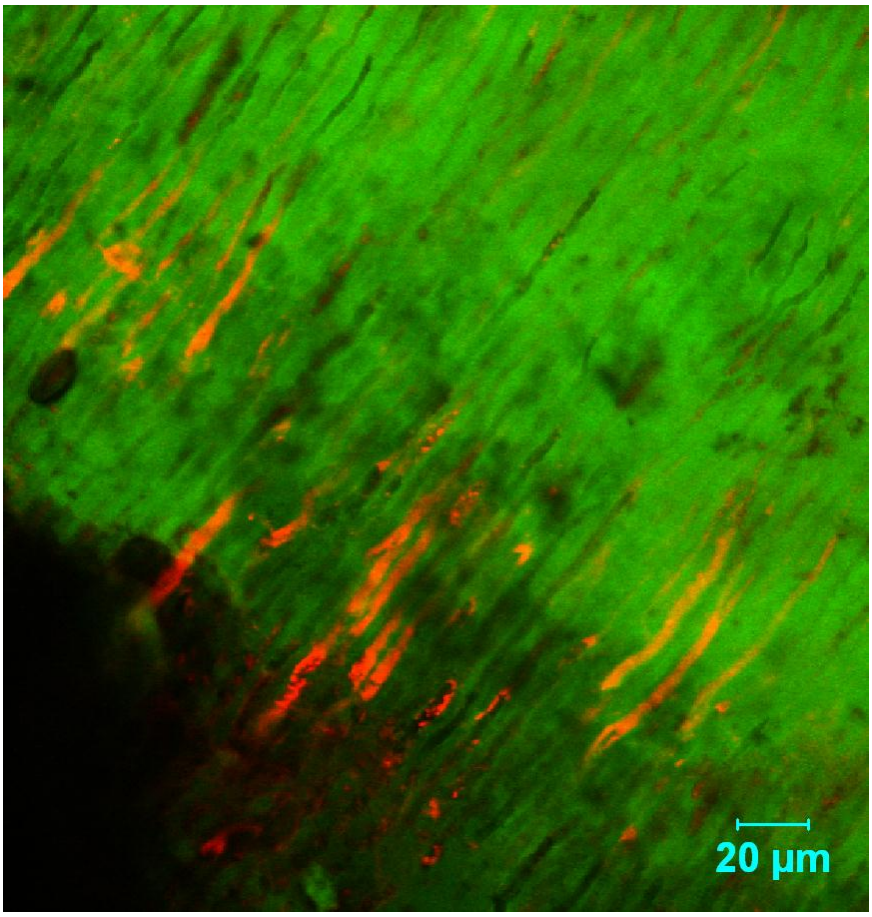


Figure 16: Anti E.faecalis efficacy of zinc oxide eugenol sealer at 45 days

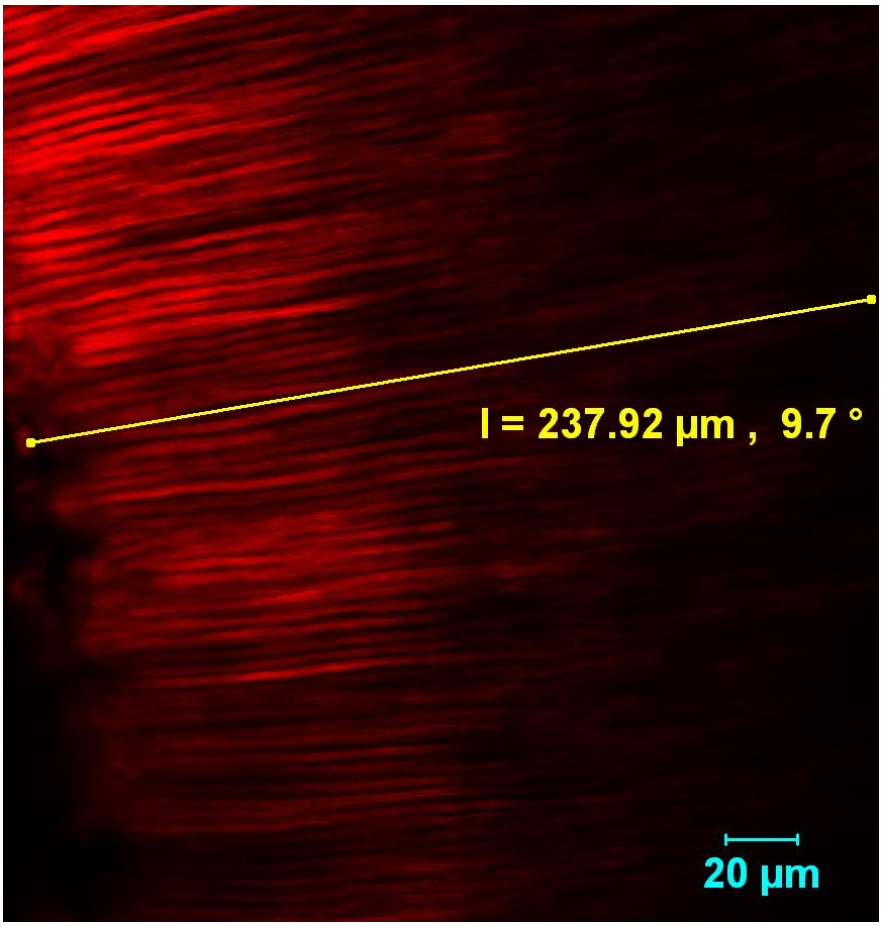


Figure 17: Depth of penetration of zinc oxide eugenol sealer (ZOE)

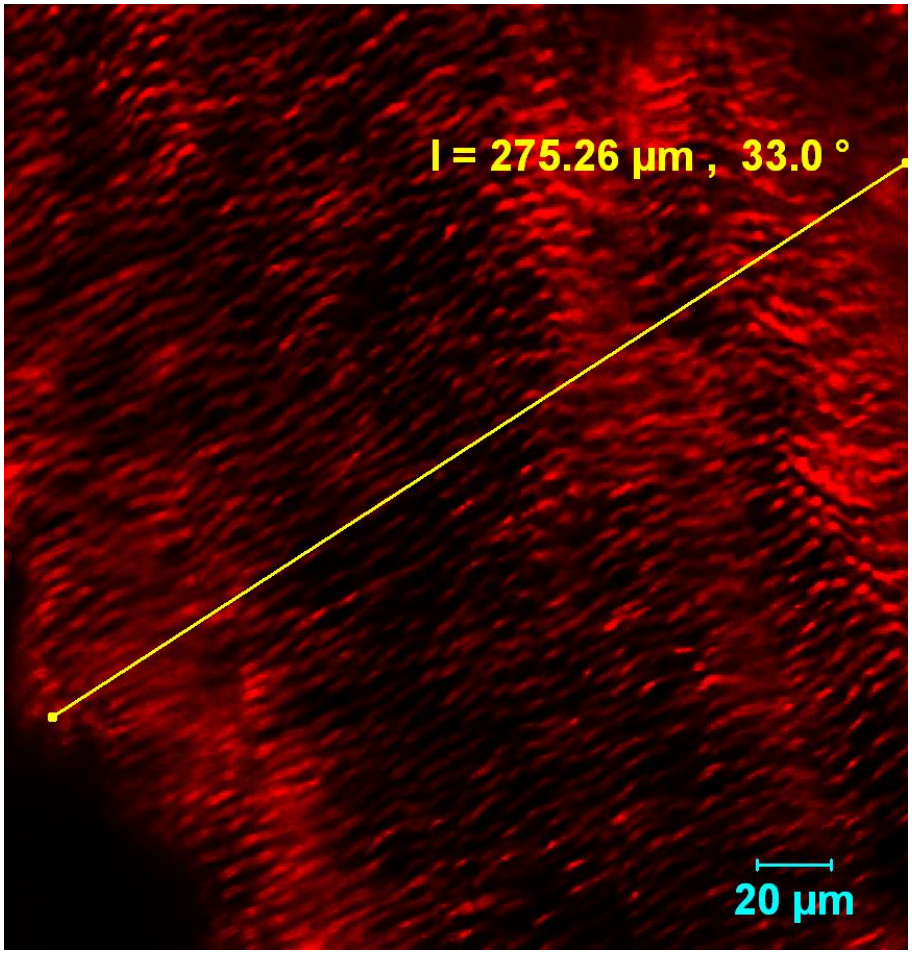


Figure 18: Depth of penetration of curcumin modified MTA sealer(MTAC)

STATISTICAL ANALYSIS

DESCRIPTIVE STATISTICS

	ZOE	MTAC	Total
Number	15	15	30
Mean	214.8373	254.0693	
Median	221.6500	262.2100	
Std. Deviation	21.21507	30.50071	

Table 4 : mean and standard deviation of depth of penetration

***Unpaired T – test for depth of penetration**

Group	ZOE	MTAC	P value*
Mean	214.8373	254.0693	0.0003
SD	21.2151	30.5007	
SEM	5.4777	7.8752	
N	15	15	

Table 5 :Comparison of depth of penetration

Mean and Standard deviation of anti E.faecalis efficacy

	ZOE	MTAC
Number	15	15
Total	**30**	
Mean	13.5667	6.2133
Median	9.4000	3.6000
Std. Deviation	12.43369	8.75858

Table 6 :Anti E.faecalis efficacy at 7 days

	ZOE	MTAC
Number	15	15
Total	30	
Mean	10.5733	2.7333
Median	5.5000	.8000
Std. Deviation	13.36148	4.94349

Table 7 :Anti E.faecalis efficacy at 21 days

	ZOE	MTAC
Number	15	15
Total	30	
Mean	.9667	3.6067
Median	.8000	2.3000
Std. Deviation	.77889	3.46358

Table 8 :Anti E.faecalis efficacy at 45 days

Unpaired T – test for anti E.faecalis efficacy of Zinc oxide eugenol

Group	ZOE 7 DAYS	ZOE 21 DAYS	P value*
Mean	13.567	10.573	0.5305
SD	12.434	13.361	
SEM	3.210	3.450	
N	15	15	

Table 9 :Comparison of Anti E.faecalis efficacy of Zinc Oxide Eugenol at 7 days and 21 days

Group	ZOE 21 DAYS	ZOE 45 DAYS	P value*
Mean	10.573	0.967	0.0096
SD	13.361	0.779	
SEM	3.450	0.201	
N	15	15	

Table 10 :Comparison of Anti E.faecalis efficacy of Zinc Oxide Eugenol at 21 days and 45 days

Group	ZOE 45 DAYS	ZOE 7 DAYS	P value*
Mean	0.967	13.567	0.0005
SD	0.779	12.434	
SEM	0.201	3.210	
N	15	15	

Table 11 :Comparison of Anti E.faecalis efficacy of Zinc Oxide Eugenol at 45 days and 7 days

Unpaired T – test for anti E.faecalis efficacy of Curcumin modified MTA sealer

Group	MTAC 7 DAYS	MTAC 21 DAYS	P Value*
Mean	6.213	2.733	0.1910
SD	8.759	4.943	
SEM	2.261	1.276	
N	15	15	

Table 12 :Comparison of Anti E.faecalis efficacy of curcumin modified MTA sealer at 7 days and 21 days

Group	MTAC 21 DAYS	MTAC 45 DAYS	P Value*
Mean	2.733	3.607	0.5797
SD	4.943	3.464	
SEM	1.276	0.894	
N	15	15	

Table 13 :Comparison of Anti E.faecalis efficacy of curcumin modified MTA sealer at 21 days and 45 days

Group	MTAC 45 DAYS	MTAC 7 DAYS	P Value*
Mean	3.607	6.213	0.2929
SD	3.464	8.759	
SEM	0.894	2.261	
N	15	15	

Table 14 :Comparison of Anti E.faecalis efficacy of curcumin modified MTA sealer at 45 days and 7 days

***Unpaired T – test for anti E.faecalis efficacy between Zinc Oxide Eugenol and Curcumin modified MTA sealer**

Group	ZOE 7 DAYS	MTAC 7 DAYS	P value*
Mean	13.567	6.213	0.0716
SD	12.434	8.759	
SEM	3.210	2.261	
N	15	15	

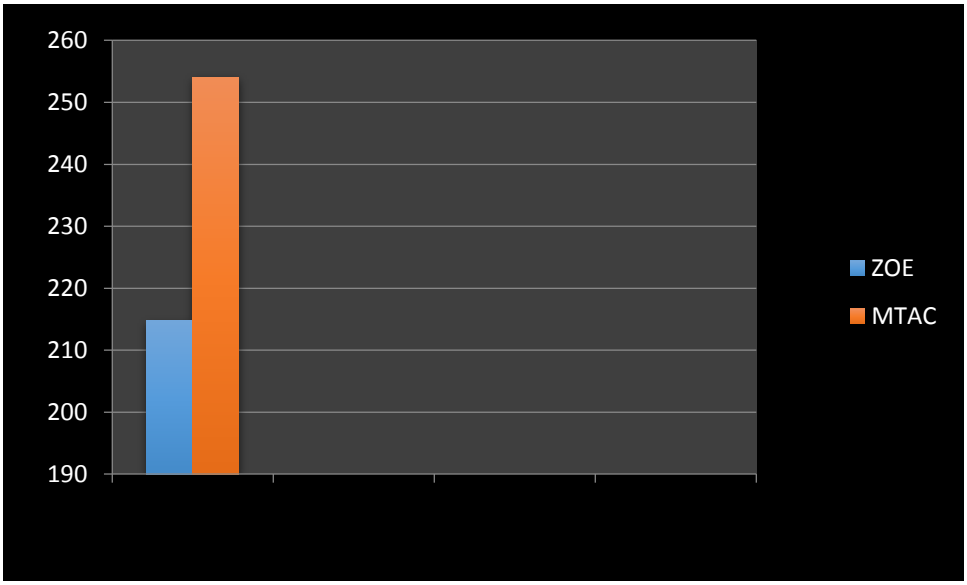
Table 15 :Comparison of Anti E.faecalis efficacy at 7 days

Group	ZOE 21 DAYS	MTAC 21 DAYS	P value*
Mean	10.573	2.733	0.0420
SD	13.361	4.943	
SEM	3.450	1.276	
N	15	15	

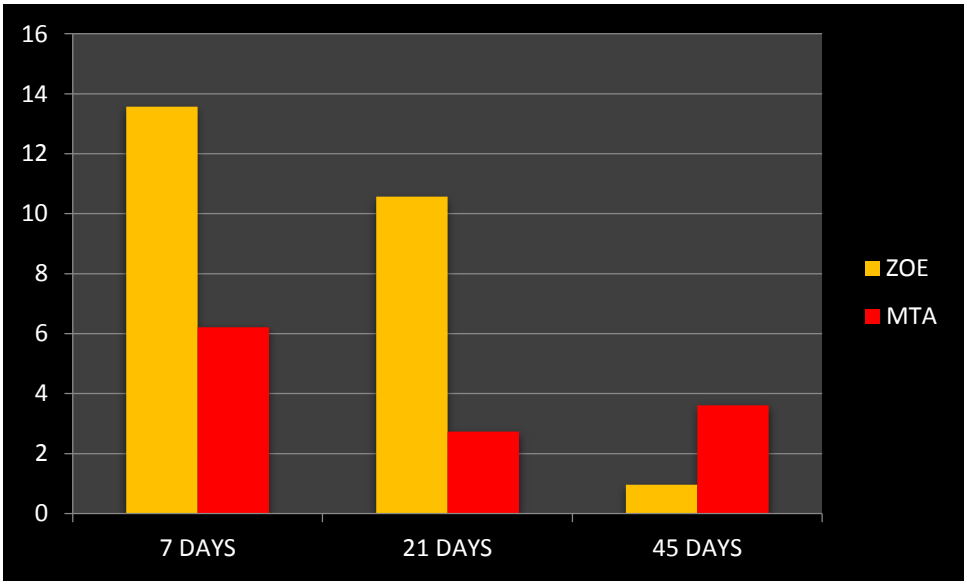
Table 16:Comparison of Anti E.faecalis efficacy at 21 days

Group	ZOE 45 DAYS	MTAC 45 DAYS	P value*
Mean	0.967	3.607	0.0075
SD	0.779	3.464	
SEM	0.201	0.894	
N	15	15	

Table 17:Comparison of Anti E.faecalis efficacy at 45 days



Line diagram 1: Depth of penetration of root canal sealers in µm



Line diagram 2 : Anti bacterial efficacy of sealer

DEPTH OF PENETRATION

The results of the present study showed that the curcumin modified MTA sealer (MTAC) had significant greater depth of penetration than ZOE. (p value is <.05;unpaired t test)

ANTI E.FAECALIS EFFICACY

The results of the present study showed that there was significantly greater anti bacterial efficacy at 7 days for ZOE than MTAC. There was significantly greater anti bacterial efficacy at 21 days for ZOE than MTAC (p value<0.05;unpaired t test). During 45 days time period , there was significantly greater anti bacterial efficacy for MTAC than ZOE.(p value<0.05;unpaired t test).

Zinc Oxide Eugenol Group (ZOE)

When comparing within groups there was no statistical significant difference between 7 days and 21 days.(p value >0.05;unpaired t test).The 21 days group showed significantly greater anti bacterial efficacy when compared to 45 days.(p value<0.05). The 7 days group showed significantly greater anti bacterial efficacy when compared to 45 days group.(p value<0.05).

Curcumin modified MTA sealer Group (MTAC)

When comparing within groups, there was no statistically significant difference between 7 days, 21 days and 45 days though the mean score showed difference.(p value>0.05;unpaired t test).

DISCUSSION

The major goal of root canal filling is to prevent any interchange between the oral cavity, the root canal system and the peri radicular tissues, thus providing a barrier to canal infection and re infection.

The ideal outcome in root canal obturation is to have a high volume of gutta-percha and a minimal volume of sealer within the body of the root canal space[53,54]and enhanced penetration into the canal irregularities and dentinal tubules. The penetration of sealers into dentinal tubules may be biologically beneficial, because laboratory studies have shown that endodontic sealers can exert antibacterial effects against bacteria in infected dentinal tubules[3]. Bacterial penetration into dentinal tubules may reach 100-1,000 μm and it can be enhanced by the absence of smear layer[28]. Many species seen in the infection of the root canal have the propensity to penetrate deeply into the dentinal tubules, such as facultative and anaerobic species[26],even close to the dentinal-cementum junction[55]. Even though there is no direct evidence to support the efficacy of sealer penetration into the tubules to kill bacteria in vivo, this achievement would seem to be reasonable especially in teeth with long standing necrotic pulps and chronic apical periodontitis. Even if the bacteria that may remain in the dentinal tubules were not killed, the sealer would serve as a reasonable blocking agent that may prevent bacterial repopulation or inactivate them in the tubules if some level of leakage in the main body of the obturated root canal occurs[46].

The influencing factor for the selection of a sealer for a successful obturation depends on the ability of a sealer to penetrate into the dentinal tubules. Since the accessory canals in apical 3 to 6 mm may communicate with the periodontal membrane they can create a periodontic endodontic pathway for potential bacterial penetration to

and from the periodontium[66,67]. Hence the depth of penetration of 2mm section from apical third of the sample was analysed.

Confocal laser scanning microscopy offers advantages compared with scanning electron microscopy and other methodologies to evaluate penetration and interfacial adaptation of root canal sealers. Visualization of the depth of penetration and adaptation of the sealers in horizontal sections is evident at low magnifications by the presence of rhodamine B fluorescence in dentinal tubules. Therefore, a panoramic vision of sealer adaptation into the root canal and dentinal tubules can be easily confirmed at higher magnifications. As indicated in earlier studies using CLSM, labeling with rhodamine B is essential to observe the extent of sealer adaptation and penetration[56,57] .

The intensity of the fluorescence in dentinal tubules is related to the quantity of sealer inside the dentinal tubule. A higher fluorescence was related to complete obturation of dentinal tubules, whereas a lower fluorescence corresponded to partial or incomplete obturation of the dentinal tubule lumen[46].

The detection of bacteria in the current study was done by using viability staining and CLSM. Viability staining has become a widely used method in measuring bacterial killing in biofilms[59-61], allowing for the measurement of the proportion of killed bacteria in each specimen, which has not been possible at the same level of sensitivity using culture methods and colony-forming unit counting. Parmar et al found that green and red fluorescent bacteria were visible within the dentinal tubules of infected root sections when examined by CLSM[62]. When using CLSM, it is inevitable that background fluorescence is also collected when dentin is examined. However, in the present model, the dentin canals were packed with high numbers of bacteria and therefore, the fluorescent signal from the bacteria was much stronger than the background fluorescence

from dentin as shown in a previous study[32] . A strong fluorescent signal from bacteria allows the use of low gain settings during confocal laser scanning microscopic scanning, minimizing the interference from background fluorescence.

Enterococcus faecalis, an opportunistic, facultative anaerobe is associated with persistent apical periodontitis in endodontically treated teeth and is highly prevalent in failed root filled teeth[68]. Survival and virulence factors of E.faecalis endures prolonged periods of nutritional deprivation, binds to dentin and proficiently invades dentinal tubules, alters host responses, suppresses the action of lymphocytes, possesses lytic enzymes, cytolysin, aggregation substance, pheromones, and lipoteichoic acid[36]. Apart from this, E. faecalis utilizes serum as a nutritional source, resists intracanal medicaments, maintains pH homeostasis and competes with other cells.

Orstavik stated that an ideal root canal sealer should have anti microbial property[6].Hence, the anti bacterial efficacy of sealer against E.faecalis is an important consideration in the selection criteria of a sealer.

The MTA sealer (MTA plus) was selected in this study due to its potential physical properties which is an another criteria for selection of a sealer.[64]

White MTA sealer did not have inhibition zones against E. faecalis. Similar results were reported previously by Estrela et al. (2000), who used grey MTA[13]. On the other hand, Tanomaruet al. (2008) verified that white MTA and Endo CPM Sealer had inhibition zones of 15 and 12 mm against E. faecalis, respectively[43]. These two sealers showed visible diffusion in the agar medium, which could lead to misinterpretation of their antibacterial activity. None of the set sealers had antibacterial activity in the direct contact test(DCT). White MTA sealer allowed the survival of E. faecalis, despite their high pH. According to McHugh et al. (2004), E. faecalis is unable to live at the pH of

11.5 or greater[50]. As the pH shown by the above mentioned sealer was between 11 and 12, it can be assumed that its alkalinity was not enough to make the environment unsuitable for the survival of that microorganism. Its proton pump is probably the key factor in its resistance to alkaline agents[36] .

Curcuminoids,a biomolecule present in turmeric showed antibacterial efficacy along with other medicinal properties[63].Components of turmeric are named curcuminoids (curcumin or diferuloylmethane, demethoxycurcumin and bisdemethoxycurcumin). These components are polyphenols with a strong antioxidant function[58].Curcumin, the most important fraction, is responsible for the biological activities of turmeric. It has been hypothesized that curcumin inhibits the assembly of a protein-filamenting temperature-sensitive mutant Z (FtsZ) protofilaments and also increases the GTPase activity of FtsZ. The perturbation of the GTPase activity of FtsZ assembly is lethal to bacteria [39].

Antimicrobial components have been incorporated in root canal sealers to prevent regrowth of residual bacteria, and different inhibitory effects have been reported for various types of sealers [65].

Hence hydroalcoholic extract of curcumin was prepared as mentioned in a previous study[63] and mixed with MTA sealer to impart anti bacterial property.

The present study was performed with MTA sealer mixed with hydro alcoholic extract of curcumin and zinc oxide eugenol sealer to assess the anti E.faecalis efficacy and depth of penetration of both sealers. However, unmodified MTA sealer has not been included in the present study.

In the present study , anti bacterial efficacy of MTAC was lesser than ZOE at 7 and 21 days. This can be attributed to the fact that curcumin is unstable above pH 6.5[69]

and the pH of MTA sealer goes upto 11[70] which might have disintegrated curcumin molecule and thus imparting less anti bacterial efficacy. But at 45 days, MTAC showed better efficacy when compared to ZOE. There was no statistically significant difference between 3 time periods of MTAC. But ZOE showed significant decrease in anti bacterial efficacy over the time periods. This could be the reason for the better result for anti bacterial efficacy of MTAC at 45 days. This is in accordance to a previous study done by Zhang et al where he found out that all the sealers including zinc oxide eugenol lost its anti bacterial property after 7 days except sealapex and endorez [4]. Eugenol is the anti microbial component present in zinc oxide eugenol sealer[71,72]. Anti microbial components should be released from the sealer matrix to be effective[73]. But the anti microbial efficacy was lost as the material set[74].

When depth of penetration was taken into consideration MTAC showed statistically significant difference from ZOE. This may be explained due to the fact that bioceramic sealer has better depth of penetration which is in accordance with the study done by Mcmichael[75] where all bioceramic sealers showed greater than 80% of sealer penetration into dentinal tubules.

Within the limitations of this study, it can be concluded that curcumin modified MTA sealer showed greater depth of penetration and sustained anti microbial efficacy for longer duration than zinc oxide eugenol.

SUMMARY

The present study was conducted in the department of conservative dentistry and endodontics, KSR institute of dental science and research.90 single rooted teeth were selected and sectioned as per the protocol mentioned by Ma et al. MTA sealer was mixed with hydroalcoholic extract of curcumin(n=45) and was placed inside the canal. Antibacterial efficacy was evaluated under CLSM (Confocal laser scanning microscope) comparing with zinc oxide eugenol.(n=45) at three different time periods(7,21 and 45 days)[n=15] using LIVE/DEAD staining procedure.

30 single rooted teeth was selected and obturated using curcumin modified MTA sealer(MTAC)(n=15) and zinc oxide eugenol(ZOE)(n=15). Rhodamine B dye was mixed with sealers to evaluate the depth of penetration under CLSM

The findings of the present study can be summarized as follows.

1) Regarding anti bacterial efficacy against E.faecalis there was no statistically significant difference for MTAC at 7, 21 and 45 days. While ZOE showed statistically significant decrease in antibacterial efficacy through out the time period from 7to 45 days.

2) The MTAC group showed greater depth of penetration at the apical third of the canal than ZOE group.

CONCLUSION

The following inference has been derived from this study. Although curcumin modified MTA sealer showed no statistically significant difference at 7, 21 and 45 days, the anti bacterial efficacy remained same through out the time period , where as the anti bacterial efficacy of zinc oxide eugenol(ZOE) was diminishing significantly from 7 to 45 days.

Curcumin modified MTA sealer showed greater depth of penetration at the apical third than zinc oxide eugenol(ZOE). With both these properties combined, it can be concluded that curcumin modified MTA sealer(MTAC) can be used as a potential root canal sealer. Further studies has to be conducted to evaluate the change in physical properties when curcumin extract is mixed with MTA sealer.

BIBLIOGRAPHY

1. Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surg, Oral Med, Oral Path. 1965 Sep 30;20(3):340-9.
2. Sundqvist G. Ecology of the root canal flora. J endod. 1992 Sep 30;18(9):427-30.
3. Saleh IM, Ruyter IE, Haapasalo M, Ørstavik D. Survival of Enterococcus faecalis in infected dentinal tubules after root canal filling with different root canal sealers in vitro. Int Endod J. 2004 Mar 1;37(3):193-8.
4. Zhang H, Shen Y, Ruse ND, Haapasalo M. Antibacterial activity of endodontic sealers by modified direct contact test against Enterococcus faecalis. J endod. 2009 Jul 31;35(7):1051-5.
5. Baer J, Maki JS. In vitro evaluation of the antimicrobial effect of three endodontic sealers mixed with amoxicillin. J endod. 2010 Jul 31;36(7):1170-3.
6. Orstavik, D.,. Antibacterial properties of endodontic materials. Int endod J, 1988, 21(2), p.161.
7. Peters LB, Wesselink PR, Buijs JF, Van Winkelhoff AJ. Viable bacteria in root dentinal tubules of teeth with apical periodontitis. J Endod. 2001 Feb 28;27(2):76-81.
8. Nair PN, Henry S, Cano V, Vera J. Microbial status of apical root canal system of human mandibular first molars with primary apical periodontitis after "one-visit" endodontic treatment. Oral Surg, Oral Med, Oral Path, Oral Rad, and Endod. 2005 Feb 28;99(2):231-52.
9. Sjögren U, Figdor D, Persson S, Sundqvist G. Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. Int Endod J. 1997 Sep 1;30(5):297-306.

10. Spangberg L, Engström B, Langeland K. Biologic effects of dental materials: 3. Toxicity and antimicrobial effect of endodontic antiseptics in vitro. Oral Surg, Oral Med, Oral Path. 1973 Dec 31;36(6):856-71.
11. Nawal RR, Parande M, Sehgal R, Naik A, Rao NR. A comparative evaluation of antimicrobial efficacy and flow properties for Epiphany, Guttaflow and AH-Plus sealer. Int Endod J. 2011 Apr 1;44(4):307-13.
12. Morgental RD, Vier-Pelisser FV, Oliveira SD, Antunes FC, Cogo DM, Kopper PM. Antibacterial activity of two MTA-based root canal sealers. Int Endod J. 2011 Dec 1;44(12):1128-33.
13. Estrela C, Baummann LL, Estrela CRA, Silva RS, Pe´cora JD Antimicrobial and chemical study of MTA, Portland cement, calcium hydroxide paste, Sealapex and Dycal.Braz Dent J 2000 11, 3–9.
14. Al-Hezaimi K, Al-Shalan TA, Naghshbandi J, Simon JH, Rotstein I. MTA preparations from different origins may vary in their antimicrobial activity. Oral surg,Oral Med, Oral Path,Oral Rad and Endod . 2009 May 31;107(5):e85-8.
15. Ruby AJ, Kuttan G, Babu KD, Rajasekharan KN, Kuttan R. Anti-tumour and antioxidant activity of natural curcuminoids. Cancer letters. 1995 Jul 20;94(1):79-83.
16. Dorai T, Aggarwal BB. Role of chemopreventive agents in cancer therapy. Cancer letters. 2004 Nov 25;215(2):129-40.
17. Mishra S, Narain U, Mishra R, Misra K. Design, development and synthesis of mixed bioconjugates of piperic acid–glycine, curcumin–glycine/alanine and curcumin–glycine–piperic acid and their antibacterial and antifungal properties. Bioorganic & medicinal chemistry. 2005 Mar 1;13(5):1477-86.

18. Panchatcharam M, Miriyala S, Gayathri VS, Suguna L. Curcumin improves wound healing by modulating collagen and decreasing reactive oxygen species. Mol Cell Biol.. 2006 Oct 1;290(1-2):87-96.
19. Gupta KK, Bharne SS, Rathinasamy K, Naik NR, Panda D. Dietary antioxidant curcumin inhibits microtubule assembly through tubulin binding. FEBS Journal. 2006 Dec 1;273(23):5320-32.
20. Singh R, Chandra R. Mridula Bose and Pratibha Mehta Luthra. Current Science. 2002 Sep 25;83(6):737.
21. Niamsa N, Sittiwet C. Antimicrobial activity of Curcuma longa aqueous extract. J of Pharmacology and Toxicology. 2009;4(4):173-7.
22. Kim KJ, Yu HH, Cha JD, Seo SJ, Choi NY, You YO. Antibacterial activity of Curcuma longa L. against methicillin-resistant Staphylococcus aureus. Phytotherapy Res. 2005 Jul 1;19(7):599-604.
23. Park BS, Kim JG, Kim MR, Lee SE, Takeoka GR, Oh KB, Kim JH. Curcuma longa L. constituents inhibit sortase A and Staphylococcus aureus cell adhesion to fibronectin. J Agric Food Chem. 2005 Nov 16;53(23):9005-9.
24. Gomes BP, Pinheiro ET, Sousa EL, Jacinto RC, Zaia AA, Ferraz CC, de Souza-Filho FJ. Enterococcus faecalis in dental root canals detected by culture and by polymerase chain reaction analysis. Oral surg,Oral Med, Oral Path,Oral Rad and Endod . 2006 Aug 31;102(2):247-53.
25. Kayaoglu G, Ørstavik D. Virulence factors of Enterococcus faecalis: relationship to endodontic disease. Crit Rev Oral Bio & Med. 2004 Sep 1;15(5):308-20..
26. Love RM. Enterococcus faecalis–a mechanism for its role in endodontic failure. Int Endod J. 2001 Jul 1;34(5):399-405.

27. Gutmann JL. Adaptation of injected thermoplasticized gutta-percha in the absence of the dentinal smear layer. Int Endod J. 1993 Mar 1;26(2):87-92.
28. Haapasalo M, Ørstavik D. In vitro infection and of dentinal tubules. J Den Res. 1987 Aug 1;66(8):1375-9.
29. Nagayoshi M, Kitamura C, Fukuizumi T, Nishihara T, Terashita M. Antimicrobial effect of ozonated water on bacteria invading dentinal tubules. J Endod. 2004 Nov 30;30(11):778-81.
30. Heling I, Chandler NP. The antimicrobial effect within dentinal tubules of four root canal sealers. J Endod. 1996 May 31;22(5):257-9.
31. Zapata RO, Bramante CM, de Moraes IG, Bernardineli N, Gasparoto TH, Graeff MS, Campanelli AP, Garcia RB. Confocal laser scanning microscopy is appropriate to detect viability of Enterococcus faecalis in infected dentin. J Endod. 2008 Oct 31;34(10):1198-201.
32. Ma J, Wang Z, Shen Y, Haapasalo M. A new noninvasive model to study the effectiveness of dentin disinfection by using confocal laser scanning microscopy. J Endod. 2011 Oct 31;37(10):1380-5.
33. Wang Z, Shen Y, Haapasalo M. Effectiveness of endodontic disinfecting solutions against young and old Enterococcus faecalis biofilms in dentin canals. J Endod. 2012 Oct 31;38(10):1376-9.
34. Du T, Wang Z, Shen Y, Ma J, Cao Y, Haapasalo M. Effect of long-term exposure to endodontic disinfecting solutions on young and old Enterococcus faecalis biofilms in dentin canals. J Endod. 2014 Apr 30;40(4):509-14.
35. Wang Z, Shen Y, Ma J, Haapasalo M. The effect of detergents on the antibacterial activity of disinfecting solutions in dentin. J Endod. 2012 Jul 31;38(7):948-53.

36. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. Enterococcus faecalis: its role in root canal treatment failure and current concepts in retreatment. J Endod. 2006 Feb 28;32(2):93-8.

37. Gomes BP, Pinheiro ET, Sousa EL, Jacinto RC, Zaia AA, Ferraz CC, de Souza-Filho FJ. Enterococcus faecalis in dental root canals detected by culture and by polymerase chain reaction analysis. Oral surg,Oral Med, Oral Path,Oral Rad and Endod . 2006 Aug 31;102(2):247-53.

38. Kayaoglu G, Ørstavik D. Virulence factors of Enterococcus faecalis: relationship to endodontic disease. Crit Rev Oral Biol & Med. 2004 Sep 1;15(5):308-20.

39. Rai D, Singh JK, Roy N, Panda D. Curcumin inhibits FtsZ assembly: an attractive mechanism for its antibacterial activity. Biochem J. 2008 Feb 15;410(1):147-55.

40. Neelakantan P, Subbarao C, Sharma S, Subbarao CV, Garcia-Godoy F, Gutmann JL. Effectiveness of curcumin against Enterococcus faecalis biofilm. Acta Odont Scand. 2013 Nov 1;71(6):1453-7.

41. Mandroli, Praveenkumar S; Bhat, Kishor, An in-vitro evaluation of antibacterial activity of curcumin against common endodontic bacteria J App Pharma Sci Oct 2013 Volume 3 Issue 10 Pages 106-108

42. Ørstavik DA. Materials used for root canal obturation: technical, biological and clinical testing. Endod Topics. 2005 Nov 1;12(1):25-38.

43. Tanomaru-Filho M, Tanomaru JM, Barros DB, Watanabe E, Ito IY. In vitro antimicrobial activity of endodontic sealers, MTA-based cements and Portland cement. J oral sci. 2007;49(1):41-5.

44. Chandra SS, Shankar P, Indira R. Depth of penetration of four resin sealers into radicular dentinal tubules: a confocal microscopic study. J Endod. 2012 Oct 31;38(10):1412-6..

45. Torabinejad M, Hong CU, Ford TP, Kettering JD. Antibacterial effects of some root end filling materials. J Endod. 1995 Aug 31;21(8):403-6.

46. Ordinola-Zapata R, Bramante CM, Graeff MS, del Carpio Perochena A, Vivan RR, Camargo EJ, Garcia RB, Bernardineli N, Gutmann JL, de Moraes IG. Depth and percentage of penetration of endodontic sealers into dentinal tubules after root canal obturation using a lateral compaction technique: a confocal laser scanning microscopy study. Oral surg,Oral Med, Oral Path,Oral Rad and Endod . 2009 Sep 30;108(3):450-7.

47. Zhang H, Shen Y, Ruse ND, Haapasalo M. Antibacterial activity of endodontic sealers by modified direct contact test against Enterococcus faecalis. J Endod. 2009 Jul 31;35(7):1051-5..

48. Miyagak DC, Carvalho EM, Robazza CR, Chavasco JK, Levorato GL. In vitro evaluation of the antimicrobial activity of endodontic sealers. Braz oral Res. 2006 Dec;20(4):303-6.

49. Sipert CR, Hussne RP, Nishiyama CK, Torres SA. In vitro antimicrobial activity of fill canal, sealapex, mineral trioxide aggregate, Portland cement and endorez. Int Endod J. 2005 Aug 1;38(8):539-43.

50. McHugh CP, Zhang P, Michalek S, Eleazer PD. pH required to kill Enterococcus faecalis in vitro. J Endod. 2004 Apr 30;30(4):218-9.

51. Al-Hezaimi K, Al-Shalan TA, Naghshbandi J, Simon JH, Rotstein I. MTA preparations from different origins may vary in their antimicrobial activity. Oral surg,Oral Med, Oral Path,Oral Rad and Endod . 2009 May 31;107(5):e85-8.

52. Holt DM, Watts JD, Beeson TJ, Kirkpatrick TC, Rutledge RE. The anti-microbial effect against enterococcus faecalis and the compressive strength of two types of

mineral trioxide aggregate mixed with sterile water or 2% chlorhexidine liquid. J Endod. 2007 Jul 31;33(7):844-7.

53. Peters DD. Two-year in vitro solubility evaluation of four gutta-percha sealer obturation techniques. J Endod. 1986 Dec 31;12(4):139-45.
54. De-Deus G, Coutinho-Filho T, Reis C, Murad C, Paciornik S. Polymicrobial leakage of four root canal sealers at two different thicknesses. J Endod. 2006 Oct 31;32(10):998-1001.
55. Peters LB, Wesselink PR, Buijs JF, Van Winkelhoff AJ. Viable bacteria in root dentinal tubules of teeth with apical periodontitis. J Endod. 2001 Feb 28;27(2):76-81.
56. D'Alpino PH, Pereira JC, Svizero NR, Rueggeberg FA, PashleyDH .Use of fluorescent compounds in assessing bonded resin based restorations: a literature review. J Dent 2006;34:623-34
57. Bitter K, Paris S, Martus P, Schartner R, Kielbassa AM. A confocal laser scanning microscope investigation of different dental adhesives bonded to root canal dentine. Int Endod J. 2004 Dec 1;37(12):840-8.
58. Chattopadhyay I, Biswas K, Bandyopadhyay U, Banerjee RK. Turmeric and curcumin: Biological actions and medicinal applications. Curr Sci. 2004 Jul 10;87(1):44-53.
59. Hamama HH, Yiu CK, Burrow MF. Viability of intratubular bacteria after chemomechanical caries removal. J Endod. 2014 Dec 31;40(12):1972-6..
60. Shen Y, Stojicic S, Haapasalo M. Bacterial viability in starved and revitalized biofilms: comparison of viability staining and direct culture. J Endod. 2010 Nov 30;36(11):1820-3.

61. Shen Y, Stojicic S, Haapasalo M. Antimicrobial efficacy of chlorhexidine against bacteria in biofilms at different stages of development. J Endod. 2011 May 31;37(5):657-61..

62. Parmar D, Hauman CH, Leichter JW, McNaughton A, Tompkins GR. Bacterial localization and viability assessment in human ex vivo dentinal tubules by fluorescence confocal laser scanning microscopy. Int Endod J. 2011 Jul 1;44(7):644-51.

63. Hegde MN, Shetty S, Yelapure M, Patil A. Evaluation of antimicrobial activity of aqueous and hydro-alcoholic Curcuma longa extracts against endodontic pathogens. IOSR J Pharma. 2012 Mar;2(2):192-8.

64. Zhang W, Li Z, Peng B. Ex vivo cytotoxicity of a new calcium silicate–based canal filling material. Int Endod J. 2010 Sep 1;43(9):769-74.

65. Lai CC, Huang FM, Yang HW, Chan Y, Huang MS, Chou MY, Chang YC. Antimicrobial activity of four root canal sealers against endodontic pathogens. Clin oral invest. 2001 Dec 1;5(4):236-9.

66. Cohen S, Burns EC. Pathways of the Pulp. 5th ed. St Louis: Mosby-Year Book, Inc; 1991. p.215-6.

67. Solomon C, Chalfin H, Kellert M, Weseley P. The endodontic-periodontal lesion: a rational approach to treatment. The J Amer Dent Assoc. 1995 Apr 30;126(4):473-9.

68. Zehnder M, Guggenheim B. The mysterious appearance of enterococci in filled root canals. Int Endod J. 2009 Apr 1;42(4):277-87.

69. Zhao C, Liu Z, Liang G. Promising curcumin-based drug design: mono-carbonyl analogues of curcumin (MACs). Curr pharmac des. 2013 Apr 1;19(11):2114-35.

70. Morgental RD, Vier-Pelisser FV, Oliveira SD, Antunes FC, Cogo DM, Kopper PM. Antibacterial activity of two MTA-based root canal sealers. Int Endod J. 2011 Dec 1;44(12):1128-33.

71. Mickel AK, Nguyen TH, Chogle S. Antimicrobial activity of endodontic sealers on Enterococcus faecalis. J Endod. 2003 Apr 30;29(4):257-8.

72. Abdulkader A, Duguid R, Saunders EM. The antimicrobial activity of endodontic sealers to anaerobic bacteria. Int Endod J. 1996 Jul 1;29(4):280-3.

73. Siqueira JF, Favieri A, Gahyva SM, Moraes SR, Lima KC, Lopes HP. Antimicrobial activity and flow rate of newer and established root canal sealers. J Endod. 2000 May 31;26(5):274-7.

74. 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. J Endod. 2016 Nov 30;42(11):1588-97.

75. . McMichael GE, Primus CM, Opperman LA. Dentinal Tubule Penetration of Tricalcium Silicate Sealers. J Endod. 2016 Apr 30;42(4):632-6.