ASSESSMENT OF ANTIBACTERIAL ACTIVITY OF FOUR ENDODONTIC SEALERS AGAINST ENTEROCOCCUS FAECALIS BY A DIRECT CONTACT TEST – AN IN-VITRO STUDY

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DEPARTMENT OF CONSERVATIVE DENTISTRY & ENDODONTICS

CERTIFICATE

This is to certify that this dissertation entitled **"Assessment Of Antibacterial Activity Of Four Endodontic Sealers Against Enterococcus Faecalis By A Direct Contact Test – An In-vitro Study"** is a genuine work done by ***Dr.RETHI GOPAKUMAR*** under my guidance during her post graduate study period between 2008-2011.

This Dissertation is submitted to THE TAMILNADU Dr. M. G. R. MEDICAL UNIVERSTY, in partial fulfillment for the degree of **MASTER OF DENTAL SURGERY IN CONSERVATIVE DENTISTRY& ENDODONTICS -BRANCH IV.** It has not been submitted (partial or full) for the award of any other degree or diploma.

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Introduction

Introduction

Endodontics is a clinical discipline of dentistry concerned with the prevention and control of root canal infection[11]. In 1894 W. D. Miller was the first who published observations from the root canals with infected pulp space. Since that time bacteria was implicated in infections of endodontic origin. Naidorf, 1972 stated that the necrotic pulp becomes a "privileged sanctuary" for clusters of bacteria and their byproducts[11]. The endodontic microflora is typically a polymicrobial flora of gram-negative and gram-positive bacteria, dominated by obligate anaerobes. The main objective of endodontic therapy is therefore to eliminate bacteria from the infected root canal and to prevent root canal infection[38].

Of the Enterococcus species, Enterococcus faecalis is the most frequently isolated species from endodontic infections. Enterococcus faecalis is recognized as a potential human pathogen causing 12% of nosocomial infections. Enterococcus faecalis has been found occasionally in cases of primary endodontic infections [23].

The ability of Enterococcus faecalis to cause periapical disease and chronic failure of an endodontically treated tooth is due to its unique ability to

bind with the collagen of the dentinal tubule and remain viable within the tubule [23].

Enterococcus faecalis also posses certain virulence factors including lytic enzymes, cytolysin, aggregation substance, pheromones, and lipoteichoicacid. The other unique feature is its ability to adhere to the host cells and express proteins that allow it to compete with other bacterial cells, and thereby altering the host response[7].

Enterococcus faecalis as an organism has the capacity to endure prolonged periods of starvation until an adequate nutritional supply becomes available. Once available, the starved organism is able to recover by utilizing serum as a source of nutrition.

Enterococcus faecalis also forms a biofilm that helps it resist destruction by enabling the bacteria to become 1000 times more resistant to phagocytosis, antibodies and antimicrobials than non-biofilm producing microorganisms[7].

Bystrom and Sundqvist (1981, 83, 85)[5] have observed that Enterococcus faecalis which survive instrumentation and irrigation rapidly

increase in numbers inside the empty canals in the time period between appointments.

Sundquist & Figdor (1998) have recommended the use of anti-bacterial medicaments between appointments during root canal therapy of non- vital teeth to maintain the canal space sterile.

The most important property in today's scenario for an ideal root canal sealer is in addition to its ideal "sealing" properties it should also prevent reinfection and growth of any microorganisms remaining in the canal, thereby favoring periapical tissue repair[45].

Many methods have been used to assess the antimicrobial efficacy of different root canal sealers. Among the test methods the agar diffusion test was the most commonly used technique but it had many limitations as it was dependent on the diffusion ability and the physical properties of tested materials. With the introduction of direct contact test by Weiss et al in 1996, the antibacterial efficacy of the endodontic sealers was tested by measuring the effect of close contact between the test bacteria and tested material based on the kinetics of bacterial growth. Moreover it is a quantitative assay which allows insoluble materials to be tested[40].

In this in-vitro study the antimicrobial activity of four different endodontic sealers, Sealapex (Calcium hydroxide based sealer), RoekoSeal (Polydimethyl siloxane based sealer), EndoRez (Urethane dimethacrylate resin based sealer), and Tubli-Seal EWT (Zinc oxide eugenol sealer), on Enterococcus faecalis based on the direct contact test is compared at time intervals of 20 minutes, 1 day and 7 days.

Aims &objectives

The aim of this in-vitro study was to evaluate and compare the antibacterial efficacy of four endodontic sealers Sealapex (Calcium hydroxide based sealer), RoekoSeal (Polydimethyl siloxane based sealer), EndoRez (Urethane dimethacrylate resin based sealer) and Tubli-Seal EWT (Zinc oxide eugenol sealer) against the microorganism Enterococcus faecalis by means of a direct contact test at time intervals of 20mts, 1day and 7days.

The objective of this in-vitro study is that in view of the increased prevalence of facultative anaerobes in unsuccessful endodontic therapy it is postulated that the antimicrobial efficacy of root canal sealers on these microorganisms may help to eliminate residual microorganisms unaffected by the effects of both chemo-mechanical preparation and intra-canal medicaments.

Review of literature

1. **Fischer (1977)**[13] analyzed the effect of three proprietary lining materials on microorganisms in carious dentine. They found that in carious dentine, zinc oxide eugenol was a more effective antibacterial agent than calcium hydroxide.

2. **Cox et al (1978)**[10] analyzed the bactericidal potential of various endodontic materials for primary teeth. Their studies showed that zinc oxide eugenol was also an effective bactericidal agent against bacterial species like staphylococcus aureus and Streptococcus viridians. The results were apparently due to the eugenol content because zinc oxide alone had no antimicrobial activity against microorganisms.

3. **Grossman (1982**)[12] had listed eleven requirements and characteristics of a good root canal sealer and one among them was that it should be bacteriostatic or at least not encourage bacterial growth.

4. **Stevens (1983)**[44] evaluated the antimicrobial potential of calcium hydroxide as an intracanal medicament in teeth of cats. Calcium hydroxide in the form of a supernatant slurry and pulp dent paste was used in comparison with camphorated chlorophenol. They found calcium hydroxide to be effective in killing microorganisms like Enterococcus

faecalis present in the root canals when compared to camphorated chlorophenol.

5. **Bystrom et al (1985)**[2] found that for calcium hydroxide sealers to be an efficient antimicrobial agent, it should maintain a pH level greater than 12.5. As the calcium hydroxide sealers sets the pH declines to 9.14, causing it to lose its effectiveness as Enterococcus faecalis can survive at a pH below 11.5.

6. **Hume (1986)**[21] studied the pharmacologic and toxicological properties of zinc oxide eugenol and stated that in the dentin immediately beneath zinc oxide eugenol, the concentration of eugenol is sufficient to inhibit bacterial metabolism.

7. **Zuhair Z. Al- Khatib et al (1990)**[49] studied the antimicrobial effect of various endodontic sealers on Streptococcus mutans, Staphylococcus aureus and Bacteroides endodontalis. The results showed that the zinc oxide eugenol based sealers had more antimicrobial activity than either the calcium hydroxide based sealers or eucapercha.

8. **Jawetz (1995)[24]** had stated that eugenol a phenolic compound acts on microorganisms by protein denaturation whereby the protein becomes non-functional.

9. **Shalhav M. Weiss EL (1996)[40]** compared the antibacterial activity of a glass ionomer based endodontic sealer, Ketac-Endo (KE), to the commonly used ZOE-based endodontic sealer, Roth's cement (RC). It was concluded that Ketac Endo possessed a short-acting very potent and diffusible antibacterial activity, whereas Roth's cement extended its effect over 7 days after setting.

10. **Heling I Chandler NP (1996)[19]** investigated the antibacterial effectiveness of four root canal sealers (Pulp Canal Sealer EWT, Sealapex, AH 26, and Ketac-Endo) within dentinal tubules infected with E.feacalis. The authors concluded that all the sealers showed antibacterial activity at 24 hrs, except Ketac-Endo. The activity of Pulp Canal Sealer EWT was similar at 24 hr and 7 days. Sealapex had greater antibacterial effect at 7 days than it did at 24 hr. The strongest effects were demonstrated by AH 26.

11. **Weiss EI Shalvey M Fuss Z (1996)[47]** evaluated the antibacterial activity of two endodontic sealers (AH 26 & Endoflas) on Enterococcus faecalis using both agar diffusion test (ADT) and direct contact test (DCT) for 18 hours. The direct contact test showed that Endoflas was significantly, a more potent bacterial growth inhibitor than AH 26, whereas when assessed by the agar diffusion test, AH 26 was capable of producing a larger inhibition zone than Endoflas. The results demonstrated the added value of DCT in the study of the antimicrobial properties of endodontic sealers.

12. **Fuss etal (1997)[14]** studied the antibacterial activity of calcium hydroxide containing sealers and a zinc oxide based sealer on Enterococcus faecalis. The results showed that the zinc oxide eugenol based sealer showed a more potent antimicrobial activity than the calcium hydroxide sealers.

13. **Shalhav M Fuss Z Weiss (1997)[40]** compared the antibacterial activity of a glass ionomer based endodontic sealer, Ketac-Endo (KE) to the commonly used ZOE-based endodontic sealer, Roth's cement (RC). With the use of E. faecalis as a test organism, the agar diffusion test (ADT)

and direct contact test (DCT) were performed for 15 hours. It was concluded that Ketac-Endo possessed a short-acting very potent and diffusible antibacterial activity, Roth's cement extended its effect over 7 days after setting.

14. **Fuss Z Weiss EI Shalhav (1997)[14]** conducted a study to analyze the antibacterial activity of calcium hydroxide containing endodontic sealers (Sealapex & CRCS) compared to a zinc oxide eugenol containing sealer (Roth's cement) on Enterococcus faecalis by a Direct Contact Test. The study was conducted for 16 hrs. The results showed that in 1-hour-old mixture, CRCS and Roth's cement had a significantly better antimicrobial effect than Sealapex. In 24-hour-old mixtures, ZOE - based sealer showed a more potent antimicrobial activity than calcium hydroxide-containing sealers, whereas Sealapex showed a significantly better antimicrobial effect in the 7-day-old mixture. The authors concluded that the antimicrobial activity of each tested sealer changes differently with the time interval between mixing and testing, suggesting different physicochemical properties and potentially diverse clinical applications.

15. **Sundqvist G et al (1998)**[14] conducted a microbiological analysis of teeth with failed endodontic treatment and the outcome of conservative retreatment. The results showed that microbial flora was mainly of a single species of predominantly gram positive organisms. The isolates most commonly recovered were bacteria of the species of Enterococcus faecalis. The overall success rate of retreatment was 74%. They also concluded that microbial flora differed markedly in failed endodontic therapy and untreated teeth.

16. **Kaplan AE et al (1999**) [26] conducted a study to determine the in vitro antimicrobial effect of six endodontic sealers after 2, 20 and 40 days by an agar diffusion test. The authors concluded that the sealers evaluated in this study showed different inhibitory effects depending on time span. Overall, sealers containing eugenol and formaldehyde proved to be most effective against the microorganisms at the time intervals studied.

17. **Zvi Fuss et al (2000)**[50] evaluated the antibacterial properties and hardness of three endodontic sealers: Roth's cement (RC), Calcibiotic Root Canal Sealer (CRCS), and AH26 with four controlled consistencies. It was concluded that endodontic sealers possess different

antibacterial and physical properties according to their mixing consistencies.

18. **Sequeira Junior JF et al (2000)[43]** investigated and compared the antimicrobial effects and the flow rate of Kerr Pulp Canal Sealer EWT, Grossman's Sealer, ThermaSeal, Sealer 26, AH Plus, and Sealer Plus. The authors concluded that these sealers have the potential to help in the microbial control in the root canal system.

19. **Leonardo MR et al (2000) [30]** evaluated the antimicrobial activity of four root canal sealers (AH Plus, Sealapex, Ketac Endo, and Fill Canal), two calcium hydroxide pastes (Calen and Calasept), and a zinc oxide paste. All bacterial strains were inhibited by all materials using the well method. However, when the materials were applied with absorbent paper points, Enterococcus faecalis was not inhibited by zinc oxide, and Pseudomonas aeruginosa was not inhibited by AH Plus, Fill Canal, and the zinc oxide-based paste.

20. **Mickel AK NguyenTH et al (2003)[35]** studied the anti bacterial activity of four endodontic sealers (Sealapex, Roth 811, Kerr EWT and AH Plus)

on Enterococcus faecalis on blood agar plates. They found no difference in the zones of inhibition between the 24 and 48 hour time period.

21. **Saleh IM. et al (2004)[38]** investigated the ability of different endodontic sealers and calcium hydroxide to kill bacteria in experimentally infected dentinal tubules The sealers tested were AH Plus (AH); Grossman's sealer (GS); Ketac-Endo (KE); Apexit (AP); Roekoseal Automix(RSA); Roekoseal Automix with an experimental primer (RP) and Calcium Hydroxide (CH). The authors concluded that root fillings in vitro with gutta-percha and AH Plus or Grossman's sealer were effective in killing E. faecalis in dentinal tubules. Other endodontic sealers, as well as Calcium Hydroxide, were less effective.

22. **Brenda Paula Figueiredo de Almeida Gomes et al (2004)[4]** investigated the antimicrobial property of five endodontic sealers namely Endo Fill, Endomethasone, Endomethasone N, Sealer 26 and AH-Plus against the following microorganisms: Candida albicans, Staphylococcus aureus, Enterococcus faecalis, Streptococcus sanguis and Actinomyces naeslundii. The results, in both methodologies used, showed that immediately after manipulation, Endo-Fill and Endomethasone

demonstrated the highest antimicrobial activity. Sealer 26 demonstrated the lowest antimicrobial activity. In conclusion, none of the sealers totally inhibited the growth of the microorganisms. Furthermore, the antimicrobial activity of each sealer decreased with time and was dependent upon the microbial susceptibility.

23. **Christopher P et al (2004)[8]** conducted an in vitro study formulated to know the exact pH required to kill E.feacalis. The study tested the growth of E.faecalis at 0.5 increments from pH 9.5 to 12. The results showed that pH 10.5 and 11.0 retarded the growth of E.feacalis, whereas no growth was seen at pH 11.5 or greater. The authors concluded that a highly alkaline intracanal pH can kill or suppress growth of E.feacalis.

24. **Kont F Cenk H, Erganis O (2004)[29]** evaluated the anti bacterial activity of five different root canal sealers (Roekoseal, Ketac Endo, AH Plus, Sealapex and Sulthan) on Enterococcus faecalis by both agar diffusion test (for 24 hrs and 7 days) and direct contact test. They concluded that anti bacterial efficacy of the materials varied according to the tests used and that the technique, time, and ingredients of the tested material can affect the results of the microbiological studies.

25. **Güven Kayagolu (2004)[17]** had described the virulence factors of E. faecalis related to endodontic infection and the periradicular inflammatory response. Enterococcus faecalis is a micro-organism that can survive extreme challenges. Its pathogenicity ranges from life threatening diseases in compromised individuals to less severe conditions, such as infection of obturated root canals with chronic apical periodontitis. The most-cited virulence factors are aggregation substance, surface adhesins, sex pheromones, lipoteichoic acid, extracellular superoxide production, the lytic enzymes gelatinase and hyaluronidase, and the toxin cytolysin. These factors are associated with various stages of an endodontic infection as well as with periapical inflammation. Some products of the bacterium may also be directly linked to damage of the periradicular tissues, which may be mediated by the host response to the bacterium and its products.

26. **Kayagolu G. et al (2005)[27]** evaluated the effect of growth at pH levels from 7.1 to 9.5 on the adherence of Enterococcus faecalis to bovine serum albumin (BSA) and collagen type I. The results showed that the adhesion of Enterococcus faecalis to BSA coated surfaces decreased inversely with alkalinity of growth medium and to collagen type I coated

surfaces of bacteria grown at pH 8.0 and 8.5 was significantly greater than for those grown at pH 7.1. Thus a minor increase in pH up to 8.5 which may be a consequence of insufficient treatment with alkaline medicaments such as calcium hydroxide increases the collagen binding ability of Enterococcus faecalis in-vitro. It was concluded that this can be a critical mechanism by which Enterococcus faecalis predominates in persistent endodontic infections.

27. **Saleh IM Ruyter IE Haapasalo M Orstavik D (2004)[38]** investigated the ability of different endodontic sealers and calcium hydroxide to kill bacteria in experimentally infected dentinal tubules. The sealers tested were AH Plus (AH); Grossman's sealer (GS); Ketac-Endo (KE); Apexit (AP); Roekoseal Automix(RSA); Roekoseal Automix with an experimental primer (RP) and Calcium Hydroxide (CH). The samples were collected from the root canal, incubated onto TSB agar and the number of colony-forming units (CFU) was determined for each sample. The authors concluded that root fillings in vitro with gutta-percha and AH Plus or Grossman's sealer were effective in killing Enterococcus faecalis in dentinal tubules. Other endodontic sealers, as well as Calcium Hydroxide, were less effective.

28. **Kayagolu G H.Erten Orstavik D (2005)[28]** evaluated the effect of growth at pH levels from 7.1 to 9.5 on the adherence of Enterococcus faecalis to bovine serum albumin (BSA) and collagen type I. The results showed that the adhesion of Enterococcus faecalis to BSA coated surfaces decreased inversely with alkalinity of growth medium and to collagen type I coated surfaces of bacteria grown at pH 8.0 and 8.5 was significantly greater than for those grown at pH 7.1. Thus a minor increase in pH up to 8.5 which may be a consequence of insufficient treatment with alkaline medicaments such as calcium hydroxide increases the collagen binding ability of Enterococcus faecalis, in- vitro. It was concluded that this can be a critical mechanism by which E.feacalis predominates in persistent endodontic infections.

29. **Sipert C R et al (2005)[42]** evaluated the in vitro antimicrobial activity of Fill Canal, Sealapex, Mineral Trioxide Aggregate (MTA), Portland cement and EndoRez was evaluated on various species of microorganisms. Sealapex and Fill Canal demonstrated antimicrobial activity for all strains. For MTA and Portland cement, only E. coli was not inhibited. No antimicrobial activity was detected for EndoRez.

30. **Giuseppe Pizzoa et al (2006)[15]** investigated the antibacterial activity of four endodontic sealers: one epoxy resin sealer (AH Plus), two zinc oxide eugenol (ZOE)-based sealers (Endomethasone, Pulp Canal Sealer), and one sealer containing both ZOE and orthophenilphenol (Vcanalare). They found that the antimicrobial activity of the tested sealers depends on the time interval between mixing and testing.

31. **Arvind,VGopikrishna DKandaswamy RajanKJeyavel(2006)[3]** evaluated the antimicrobial efficacy of a traditional zinc oxide eugenol based sealer(Tubliseal) with a iodoform incorporated zinc oxide eugenol based sealer (Endoflas FS), a calcium hydroxide based sealer (Apexit) and the epoxy resin based sealers (AH PLUS and PC Seal), against the micro organisms Enterococcus faecalis and Candida albicans. Tubliseal, a zinc oxide eugenol based sealer showed significant antimicrobial properties, but was statistically inferior to Endoflas FS. Apexit, a calcium hydroxide based sealer did not show significant antimicrobial efficacy against both Enterococcus faecalis and Candida albicans. AH PLUS and RC seal, epoxy resin based sealers showed no antimicrobial properties whatsoever.

32. **Chiara Pirani1 Angelica Bertacci et al (2007)[32]** studied the presence of Enterococcus faecalis in root canals of teeth affected by primary and secondary periapical lesions using polymerase chain reaction (PCR) assays. The study confirmed the high presence of E. faecalis in secondary apical lesions. However, its effective role in endodontic pathogenesis such as bone periapical lesions needs to be clarified.

33. **Sandra B Pérez Denise P et al (2008)[39]** conducted an in- vitro study to evaluate the duration of the antimicrobial effect of endodontic sealers by means of the Direct Contact Test. The sealers tested were: Endomethasone, Septodont, Endion-Voco, Diaket-ESPE, Pulp Canal Sealer-SybronEndo, and AH26-Dentsply DeTrey. It was concluded that the structural features and virulence of endodontopathic microorganisms determine their response to the sealers, independently of the time during which sealers act and the mechanism by which the antiseptic reaches the microorganism, which in this case was by direct contact.

34. **L Smadi A Khraisat, S K Al-Tarawneh A Mahafzah, A Salem (2008)[41]** Conducted an in-vitro study to analyze the antimicrobial activity of root canal sealers by using the direct contact test. Topseal, AH

plus, AH 26, Sealite regular and Acroseal showed significant differences only when freshly mixed. It was concluded that that the antimicrobial activity of the tested sealers depends on the time interval between mixing and testing. Most sealers exhibited antibacterial activity when freshly mixed that is lost over time.

35. **Hui Zhang et al (2009)[20]** studied in vitro the antibacterial effectiveness of 7 different endodontic sealers, AH Plus, Apexit Plus, I Root SP, Tubli Seal, Sealapex, Epiphany SE, and EndoRez against Enterococcus faecalis. Fresh I Root SP, AH Plus, and EndoRez killed Enterococcus faecalis effectively. I Root SP and EndoRez continued to be effective for 3 and 7 days after mixing. Sealapex and EndoRez were the only ones with antimicrobial activity even at 7 days after mixing.

36. **Cláudia Ramos Pinheiro. Adriana Simionatto Guinesi et al (2009)[9]** using the agar diffusion method, evaluated the in *vitro* antimicrobial activity of the commercial endodontic sealers Acroseal and Epiphany, a castor-oil based experimental sealer, Polifil, and a primer agent (Epiphany self-etching primer), against Enterococcus faecalis. After 48 h, the diameters of the zones of microbial growth inhibition were the

same as those observed at 24 h, only the substances continued to diffuse. Epiphany and Polifil did not show antibacterial activity (no formation of zones of microbial growth inhibition). The primer produced the largest zones of inhibition (17.62 mm) followed by Acroseal (7.25 mm) and ZOE (7.12 mm). Enterococcus faecalis was resistant to Epiphany and Polifil, while the primer and Acroseal sealer were effective against this microorganism under the tested conditions.

37. **Jeff Baer. James S Maki (2010)[25]** conducted an in vitro study to evaluate the antimicrobial effect of mixing amoxicillin with three different sealers when freshly mixed and set using a direct contact test. Sealers mixed with amoxicillin inhibited the growth of Enterococcus faecalis significantly greater than sealers without amoxicillin ($p < 0.001$).

Materials &methods

An in-vitro study to evaluate the anti bacterial activity of four endodontic sealers on Enterococcus faecalis by a direct contact test was undertaken in the Department of Mycobacterium Research group of Rajiv Gandhi Centre for Biotechnology, Poojappura, Trivandrum.

Source of data:

Enterococcus faecalis pure strains ATCC 29212 obtained from the Department of Microbiology, Centre for Earth Science Studies, Akkulam, Trivandrum, was employed for testing the antibacterial efficacy of endodontic materials.

Method of collection of data:

Data is collected by recording the optical density, a measurement of turbidity that is based on the kinetics of bacterial growth, with the help of a Bio-Rad Microplate reader.

Study Materials:

Four commercially available root canal sealers were used in this study.

Sl:No:	Material	Trade name	Composition
1	Calcium hydroxide polymeric sealer	Sealapex (SybronEndo)	CATALYST: Isobutyl salicylate resin, fumed silica (silicon dioxide), bismuth trioxide, titanium dioxide pigment BASE : N-ethyl toluene solfanamide resin, fumed silica (silicon dioxide), zinc- oxide, calcium oxide
2	Polydimethyl siloxane based sealers	RoekoSeal (Coltene Whaledent)	Gutta percha powder Polydimethyl siloxane Silicone oil, Paraffin oil, Platin catalyst Zirconium dioxide Nano silver (preservative)
3	UDMA resin-based, root canal sealer	EndoRez (Ultradent)	Urethane dimethacrylate resin (matrix) Zinc oxide Barium Sulfate and Resin pigments
4	Zinc oxide Eugenol based radiopaque sealer	Tubli-seal EWT (SybronEndo)	ACCELERATOR: 4-Allyl-2-Methoxyphenol 97-53-0 NA 24,Dimeric acid resin and mineral oil BASE : Mineral oil, barium sulfate, zinc oxide, lecithin, cornstarch

The sealers were prepared in strict compliance with the manufacturers' recommendation.

Grouping and preparation of the specimen:

Group	Dispensing	Mixing time	Setting time
GROUP I Sealapex	1:1	15-20secs	60 mins
GROUP II Roekoseal	1:1	30secs	45 – 50 mins
GROUP III EndoRez	Uniformly mixed by an Ultra mixer tip	5 – 10 secs	15 – 20 mins
GROUP IV Tubliseal EWT	Catalyst base ratio of 1.9cm: 1.9 cm	20secs – 1 min	2hrs less than 120mts
GROUP V (Control)	The growth of the micro organism in the absence of the sealer.		

Test microorganism:

Enterococcus faecalis used for testing antimicrobial activity of endodontic materials was obtained from the Department of Microbiology, Centre for Earth Science Studies, Akkulam, Trivandrum.

Preparation of the medium for Enterococcus faecalis:

Brain Heart Infusion Broth M210-110G:

Brain Heart Infusion Broth is employed for the propagation of fastidious pathogenic cocci and other organisms associated with blood culture work and allied pathological investigations. Brain Heart Infusion Medium is useful for cultivating a wide variety of microorganisms since it is a highly nutritive medium. It is also used to prepare the inocula for antimicrobial susceptibility testing and is a modification of the original formulation of Rosenow, where pieces of brain tissues were added to dextrose broth. This medium is nutritious and well buffered to support the growth of wide variety of organisms

Composition:

Ingredients: gms / Litre

Calf brain, infusion from 200.000

Beef heart, infusion from 250.000

Protease peptone 10.000

Dextrose 2.000

Sodium chloride 5.000

Disodium phosphate 2.500

Final pH (at 25°C) 7.4±0.2

37 grams of the dehydrated media is suspended in 1000 ml distilled water. It is then dispensed into bottles or tubes and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes. For best results, the medium will have to be used on the day it is prepared, otherwise, it should be boiled or steamed for a few minutes and then cooled before use.

Preparation of specimens:

Bacteria were grown anaerobically from frozen stock cultures in brain heart infusion (BHI) broth at 37°C. Cells were harvested by centrifugation and resuspended in fresh medium. Inoculums were

prepared by the resuspension of washed cells to predetermined optical densities which relate to known concentrations.

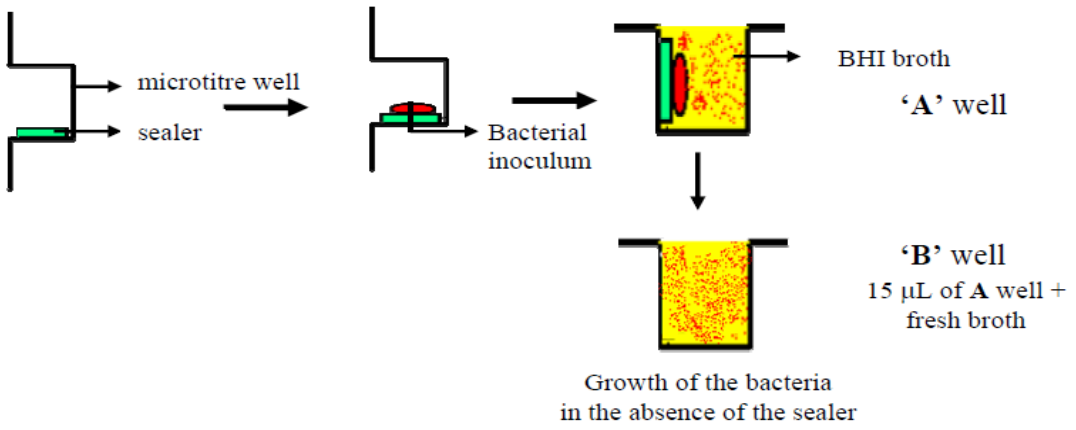
Brain Heart Infusion Agar for the culture:

7.4 gms of BHI was mixed with 3.4 gms of agar powder and mixed with 100ml of distilled water. It is then sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Brain heart infusion broth which is not used on the day that it is sterilized should be placed in a boiling water bath for several minutes to remove absorbed oxygen, and cooled rapidly without shaking just before use.

The cooled broth is then poured into Petri dishes, sealed with paraffin tapes, labeled and kept in a sterile environment at 37°c.

Direct Contact test - (DCT)

The direct contact test is based on turbidometric determination of bacterial growth in 96-well microtiter plates. The kinetics of the outgrowth in each well is monitored at 600 nm at 37^{0}C and recorded every 1 hr using a Bio-Rad microplate reader.



Schematic representation of the DCT

Of the 96 wells of a microtitre plate, 8 wells were utilized per sealer of which 4 were designated as 'A' wells (with the sealer) and the other 4 as 'B' wells (without the sealer). The 'A' wells were held vertically, i.e., the plate's surface was maintained perpendicular to the floor plane and the side wall was coated with the freshly mixed test sealer. Even and thin coating of the sealer was achieved by using a small size round ended dental instrument. Special care was taken to avoid the material's flow to the bottom of the well, which would interfere with the path of light through the micro plate well and result in false readings.

After 20 min, a 10 μL bacterial suspension (10^6 bacteria) was placed on the test material. The plate was held in a vertical position and wells were inspected for evaporation of the suspension's liquid, which occurred within 1 hr at 37°C. This ensured direct contact between bacteria and tested material. Brain Heart Infusion broth (245 μL) was added to each of these A wells and gently mixed for 2 min.

15 μL of broth was then transferred from A wells to an adjacent set of B wells containing fresh medium (215 μL). This resulted in two sets of 4 wells for each tested material containing an equal volume of liquid medium, so that bacterial out growth could be monitored both in the presence and in the absence of the tested material. Following the outgrowth of the microorganism in the presence of the sealer (Group A wells) is equivalent to measuring both the direct contact effect and the effect of those components which are capable of diffusing into the liquid medium, whereas following bacterial growth in the absence of the tested materials (Group B wells) measures the effect of the direct contact incubation period only.

Four uncoated wells in the same micro titer plate served as positive control, i.e., identical bacterial inoculum was placed on the side wall of the uncoated wells and processed as the experimental A and B wells. The plate was placed for incubation at 37°C for 1 hour and the optical density in each well was measured at 600 nm in the microplate reader. The readings were taken at regular intervals (every 1hr for 7 hours). Data were recorded, then plotted and statistically analyzed using, Kruskal Wallis One way Anova and Mann –Whitney 'U' test.

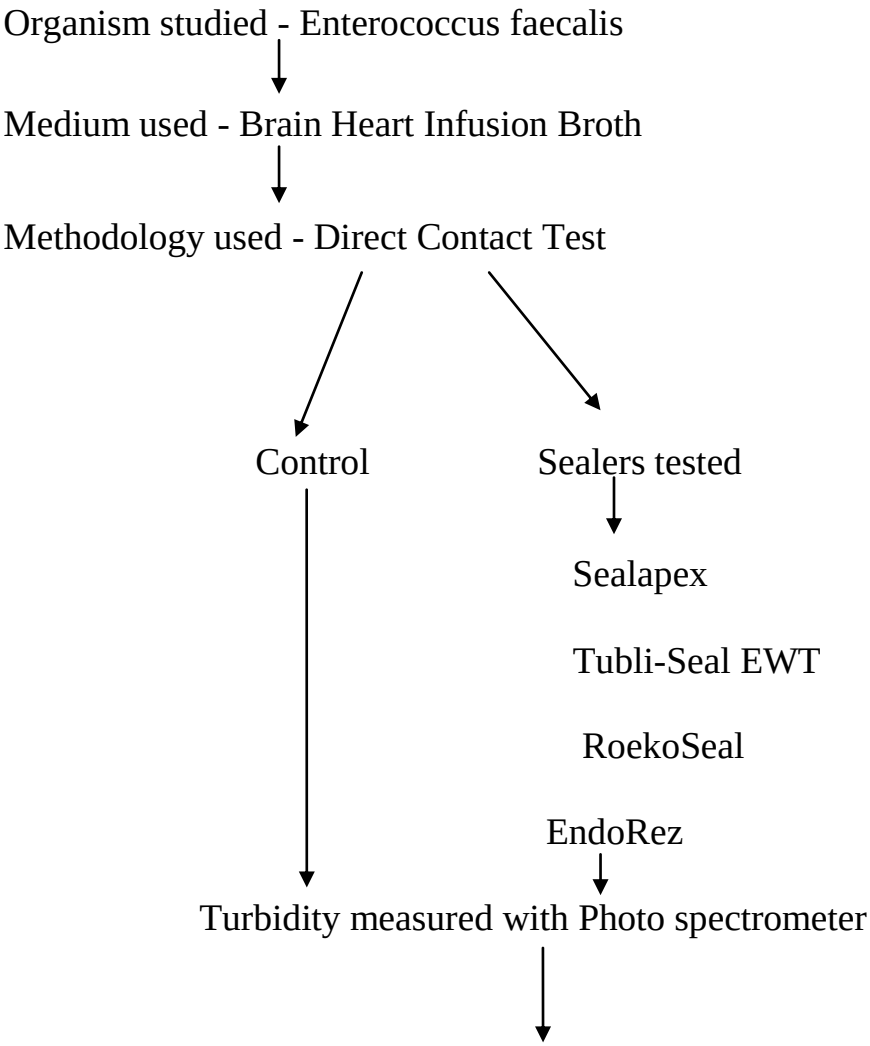
The whole experiment was carried out under aseptic conditions and was repeated three times to ensure reproducibility.

Investigation design

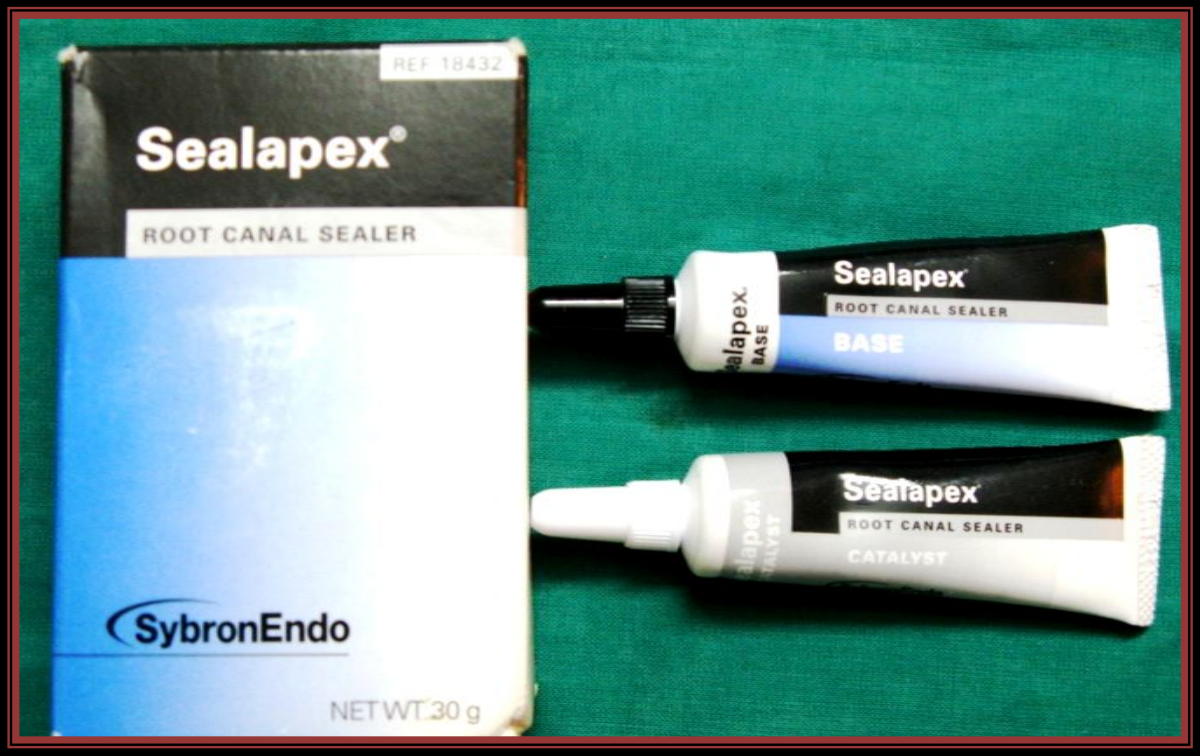
- 8 wells of a microtitre plate were utilized per each tested sealer of which 4 were designated as 'A' wells (with the sealer) and the other 4 as 'B' wells (without the sealer).
- Side wall of A wells is coated with freshly mixed tested material (endodontic sealer) according to the manufacturer instructions and allowed to set.
- 10 µL bacterial suspension is placed on tested material and incubated for an hour.
- 245 µL of brain heart infusion broth is added and gently mixed.
- 15 µL is transferred from 'A' wells to adjacent set of 4 wells containing fresh broth (215 µL) (B wells).
- A set of 4 uncoated wells in the same microtitre plate with the identical bacterial inoculum are taken as Control wells (230µL)
- The microtitre plate is incubated at 37 °C and optical density in each well is measured at regular intervals (readings are taken every 1hr for 7 hours).

- The whole experiment is repeated 3 times for each sealer to ensure reproducibility.
- Data is recorded and statistically analyzed using Kruskal Wallis One way Anova and Mann –Whitney ‘U’ test.

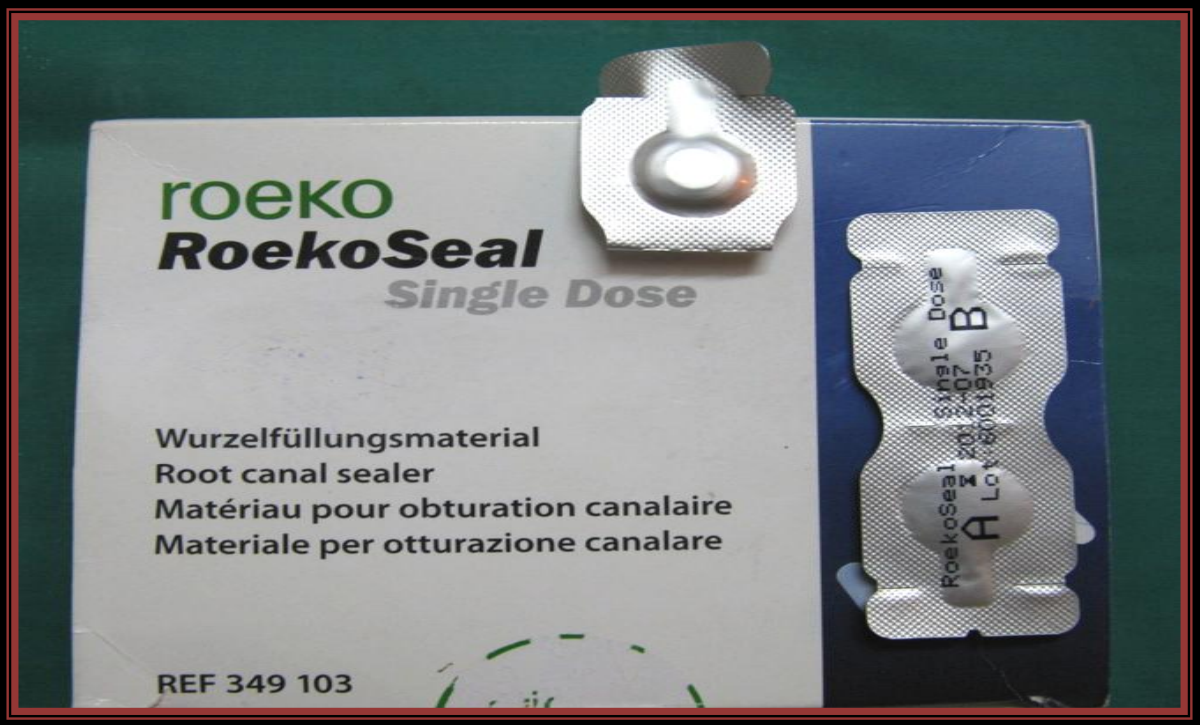
Flow chart


Organism studied - Enterococcus faecalis
Medium used - Brain Heart Infusion Broth
Methodology used - Direct Contact Test
Control
Sealers tested
Sealapex
Tubli-Seal EWT
RoekoSeal
EndoRez
Turbidity measured with Photo spectrometer
Analysis done using Kruskal Wallis One way Anova
and Mann –Whitney 'U' test.

STUDY MATERIALS



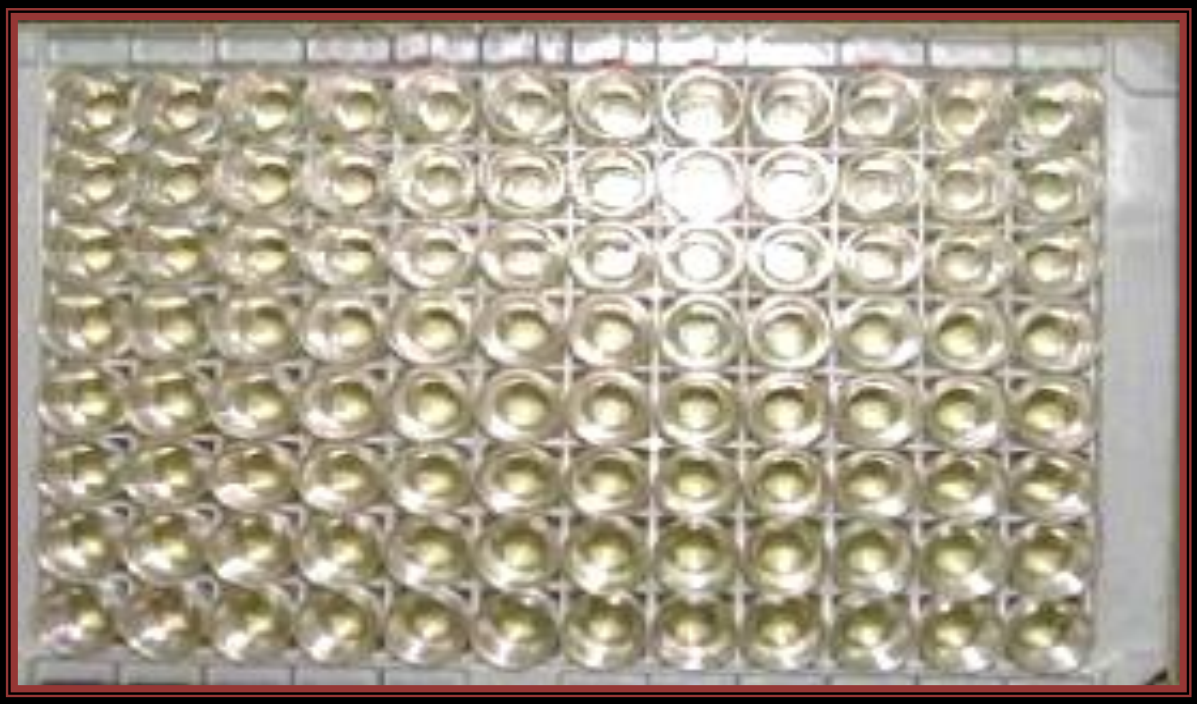
1. SEALAPEX-Calcium hydroxide based polymeric sealer



2. ROEKOSEAL— Polydimethyl siloxane based sealers

3.ENDOREZ— Urethane dimethacrylate resin-based, sealer

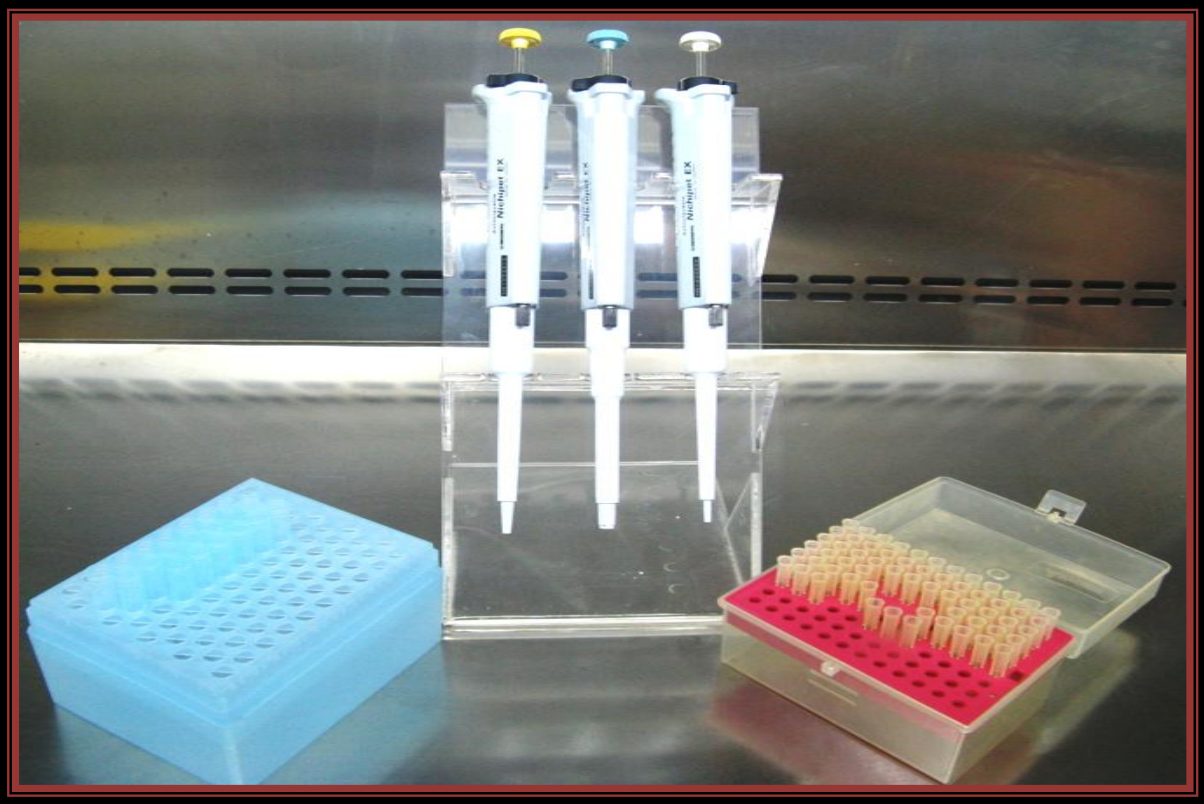
4. TUBLI-SEAL EWT - Zinc oxide Eugenol based radio opaque sealer



5. MICROTITRE PLATES WITH 96 WELLS

6. MICROTITRE WELLS OF EACH SPECIMEN

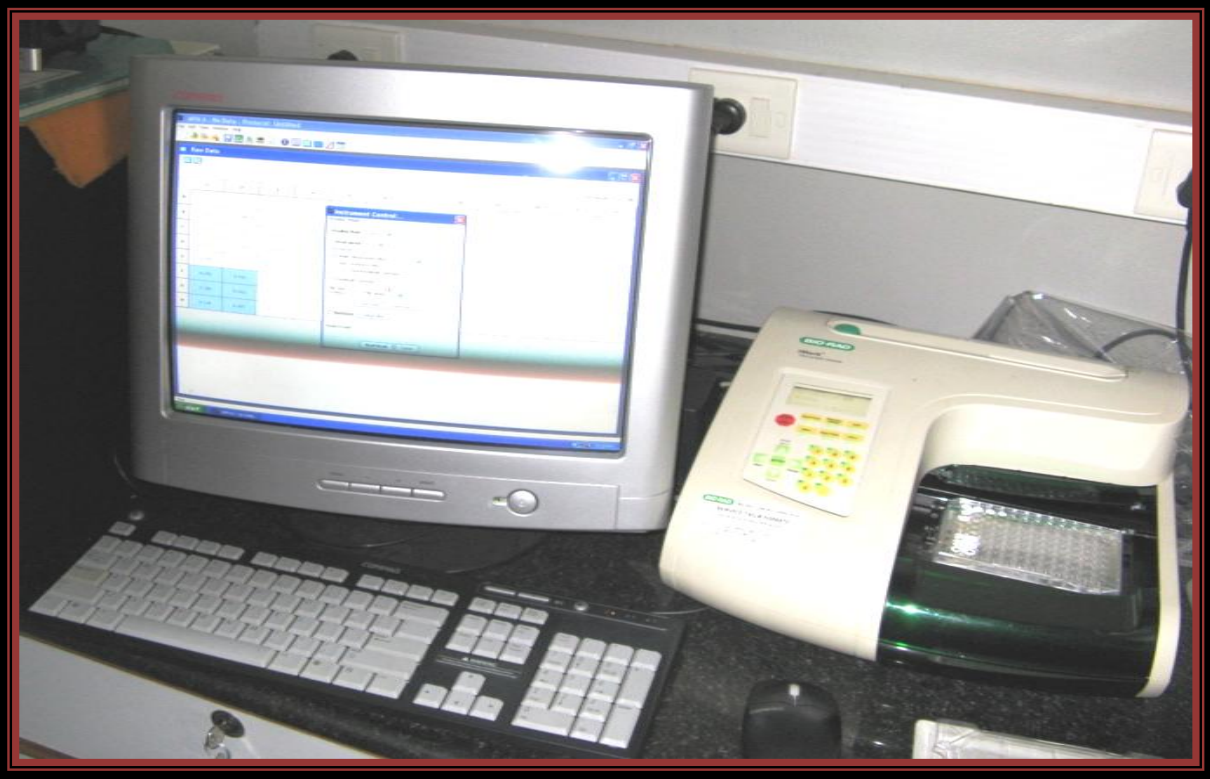
7.MICROTITRE WELLS COATED WITH SEALER (A wells)



8. MICROPIPETTE WITH TIPS

9. STERILE HOOD

10. INCUBATOR



11.PHOTOSPECTROMETER

12. BIO RAD MICROPLATE READER

Results

Data was collected by recording the optical density, a measurement of turbidity that is based on the kinetics of bacterial growth, with the help of a spectrophotometer (Bio-Rad Microplate Reader). Statistical analysis was done using Kruskal Wallis One way Anova and Mann –Whitney 'U' test.

TABLE I

STUDY GROUPS

GROUP I	Seal Apex
GROUP II	Roekoseal
GROUP III	Endo Rez
GROUP IV	Tubliseal EWT
GROUP V	Control

OBSERVATION AND RESULTS:

Table I - Group comparison using Kruskal Wallis test 20 mts after mixing.

Groups	No: of samples	Mean	Median	S.D	Kruskal Wallis H = +54.83 P= 0.0001 HS.
Group I (Sealapex)	32	0.676	0.723	0.225	
Group II (RoekoSeal)	32	0.530	0.566	0.86	
Group III (EndoRez)	32	0.386	0.376	0.081	
Group IV(Tubli-Seal EWT)	32	0.713	0.675	0.262	
Group V(Control)	32	0.315	0.271	0.153	

Table II- Group comparison using Kruskal Wallis test 1day after mixing

Groups	No: of samples	Mean	Median	S.D	Kruskal Wallis H = + 22.16 P= 0.0001 HS.
Group I (Sealapex)	32	0.781	0.862	0.270	
Group II (RoekoSeal)	32	0.561	0.469	0.285	
Group III (EndoRez)	32	0.382	0.383	0.180	
Group IV(Tubli-Seal EWT)	32	0.808	1.616	0.207	
Group V(Control)	32	0.315	0.271	0.153	

Table III- Group comparison using Kruskal Wallis test 7 days after mixing

Groups	No: of samples	Mean	Median	S.D	
Group I (Sealapex)	32	0.612	0.783	0.286	Kruskal Wallis H = + 26.72 P= 0.0001 HS.
Group II (RoekoSeal)	32	0.487	0.474	0.144	
Group III (EndoRez)	32	0.432	0.304	0.102	
Group IV(Tubli-Seal EWT)	32	0.759	0.778	0.249	
Group V(Control)	32	0.315	0.271	0.153	

Table IV- Inter group comparison using Mann Whitney 'U' test 20 mts after mixing

Groups	U	p	Inference
Group V(control) vs Group I (Sealapex)	137.00	0.0001	Hs
Group V (control) vs Group II (RoekoSeal)	270.00	0.001	S
Group V (control) vs Group III (EndoRez)	466.50	0.541	Ns
Group V (control) vs Group IV (Tubli-Seal EWT)	59.50	0.0001	Hs

U = Mann Whitney 'U' Test; S = significant; P = Probability; Ns = not significant; Hs = highly significant.

Table V- Inter group comparison using Mann Whitney 'U' test 1 day after mixing

Groups	U	p	Inference
Group V (control) vs Group I (Sealapex)	200.50	0.0001	Hs
Group V (control) vs Group II (RoekoSeal)	351.50	0.031	Ns
Group V (control) vs Group III (EndoRez)	348.50	0.028	Ns
Group V (control) vs Group IV (Tubli-Seal EWT)	183.50	0.0001	Hs

U = Mann Whitney 'U' Test; S = significant; P = Probability; Ns = not significant; Hs = highly significant.

Table VI- Inter group comparison using Mann Whitney 'U' test 7 days after mixing

Groups	U	p	Inference
Group V (control) vs Group I (Sealapex)	236.50	0.0001	Hs
Group V (control) vs Group II (RoekoSeal)	360.00	0.041	Ns
Group V (control) vs Group III (EndoRez)	413.00	0.184	Ns
Group V(control) vs Group IV (Tubli-Seal EWT)	134.00	0.0001	Hs

U = Mann Whitney 'U' Test; S = significant; P = Probability; Ns = not significant; Hs = highly significant.

Table I - Shows group comparison performed 20 mts after mixing by Kruskal Wallis analysis. Results indicate that the Group IV (Zinc Oxide Eugenol sealer) showed the maximum mean value (0.713) and Group V(Control) showed the least (0.315) suggesting highly significant difference between the groups (p=0.0001).

Table II - Shows group comparison performed 1 day after mixing by Kruskal Wallis analysis. Results indicate that the Group IV (Zinc Oxide-Eugenol sealer) showed the maximum mean value (0.808) and Group V (Control) showed the least (0.315) suggesting highly significant difference between the groups (p=0.0001).

Table III - Shows group comparison performed 7 days after mixing by Kruskal Wallis analysis. Results indicate that the Group IV (Zinc Oxide-Eugenol sealer) showed the maximum mean value (0.759) and Group V (Control) showed the least (0.315) suggesting highly significant difference between the groups (p=0.0001).

Table IV - Indicates Inter group comparison between the four groups using Mann Whitney 'U' test 20 minutes after mixing. Results indicate that in comparison to the control

Group I (Sealapex) showed P = 0 .0001, which is highly significant in comparison to control.

Group II (RoekoSeal) showed P = 0.001, which is significant in comparison to control.

Group III (EndoRez) showed P =0.541, which is not significant in comparison to control and

Group IV (Tubli-Seal EWT) showed P = 0 .0001, which is very highly significant in comparison to control.

Table V - Indicates Inter group comparison between the four groups using Mann Whitney 'U' test 1 day after mixing. Results indicate that in comparison to the control

Group I (Sealapex) showed P = 0 .0001, which is highly significant in comparison to control.

Group IV (Tubli-Seal EWT) showed P = 0 .0001, which is very highly significant in comparison to control.

Group II (RoekoSeal; P = 0.031) and Group III (EndoRez; P =0.028) did not show any significance in comparison to control.

Table VI - Indicates Inter group comparison between the four groups using Mann Whitney 'U' test 7 days after mixing. Results indicate that in comparison to the control

Group I (Sealapex) showed P = 0 .0001, which is highly significant in comparison to control.

Group IV (Tubli-Seal EWT) showed P = 0 .0001, which is very highly significant in comparison to control.

Group II (RoekoSeal; P = 0.041) and Group III (EndoRez; P =0.184) did not show any significance in comparison to control.

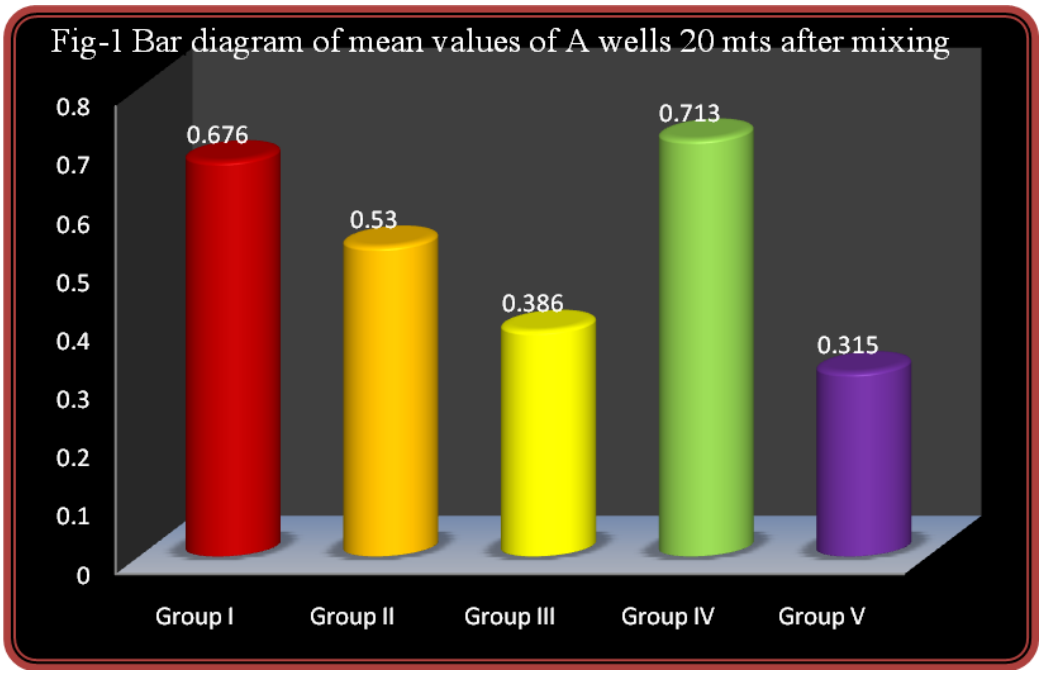


Fig-1 Bar diagram of mean values of A wells 20 mts after mixing
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.676
0.53
0.386
0.713
0.315
Group I
Group II
Group III
Group IV
Group V

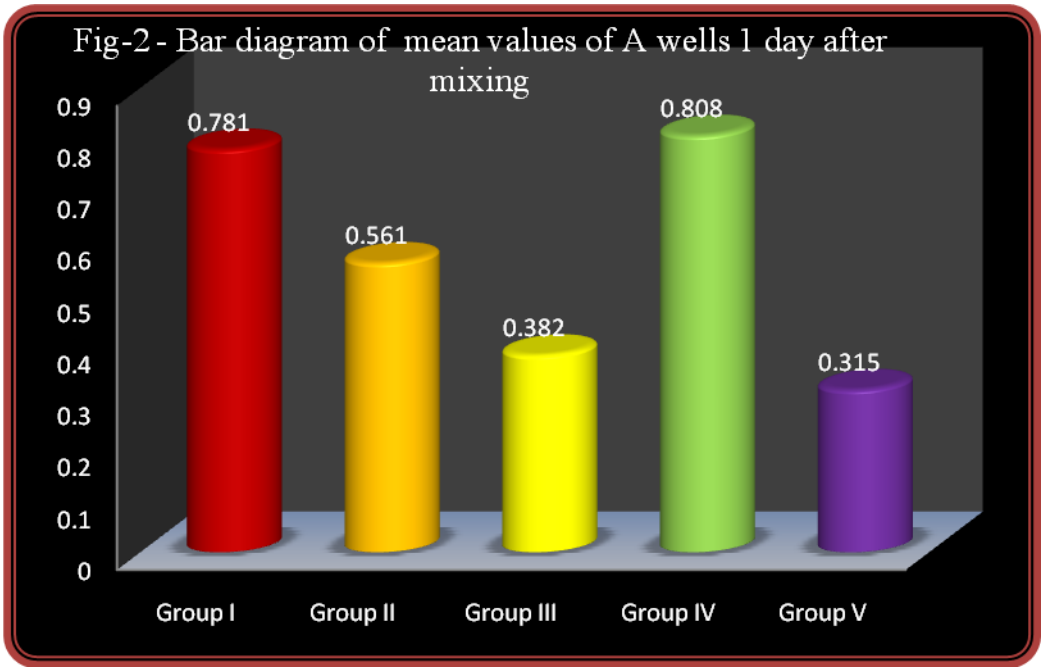


Fig-2 - Bar diagram of mean values of A wells 1 day after mixing
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.781
0.561
0.382
0.808
0.315
Group I
Group II
Group III
Group IV
Group V

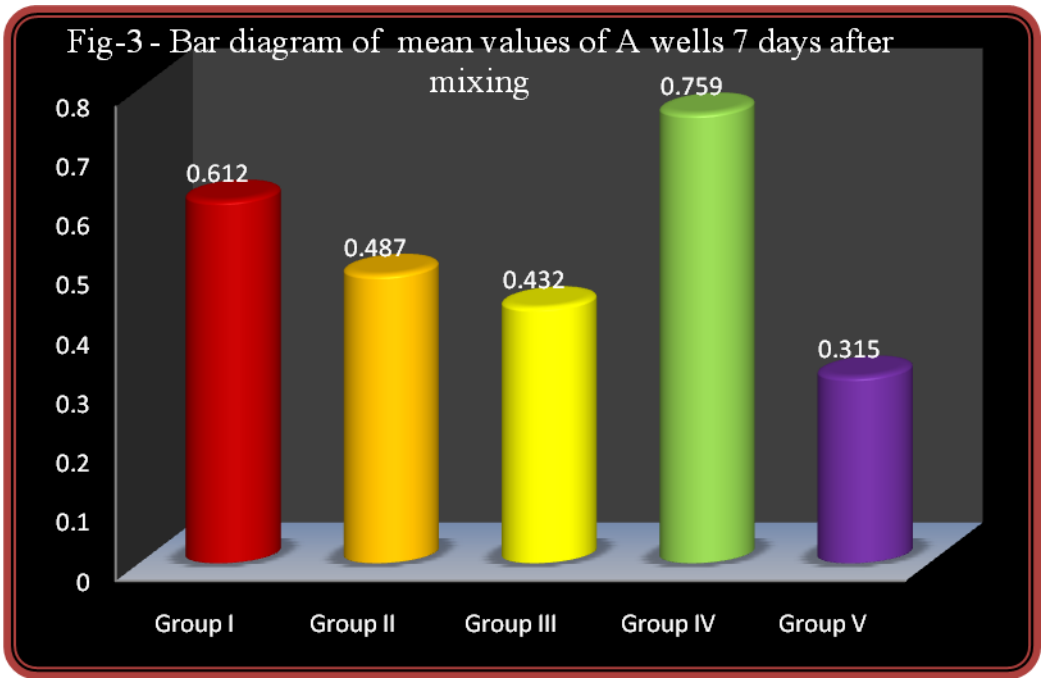


Fig-3 - Bar diagram of mean values of A wells 7 days after mixing
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.612
0.487
0.432
0.759
0.315
Group I
Group II
Group III
Group IV
Group V

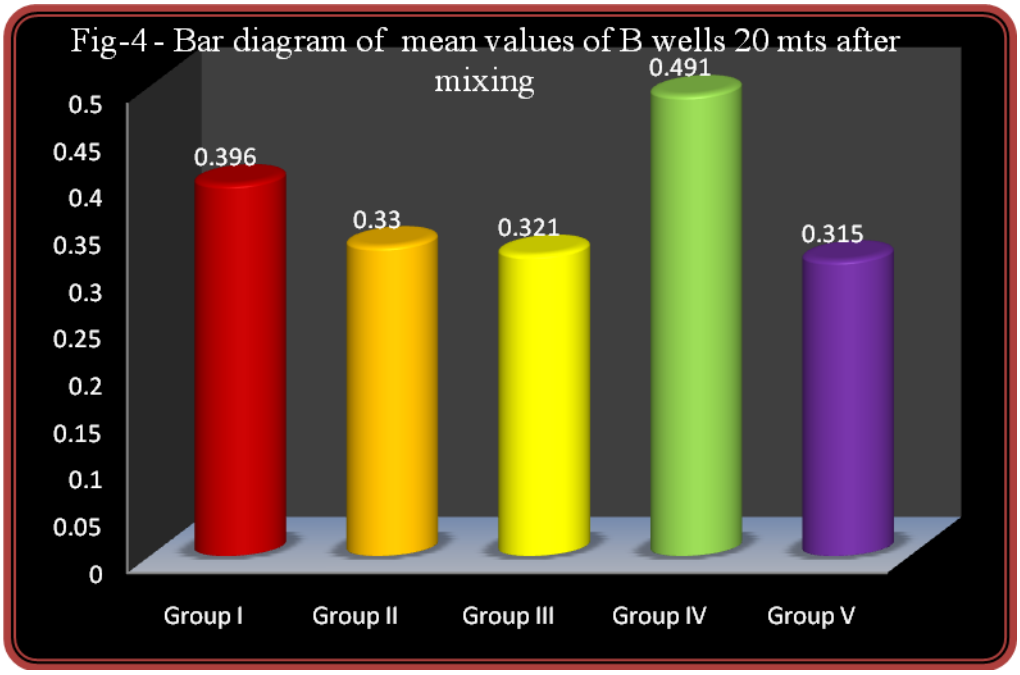


Fig-4 - Bar diagram of mean values of B wells 20 mts after mixing
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0.396
0.33
0.321
0.491
0.315
Group I
Group II
Group III
Group IV
Group V

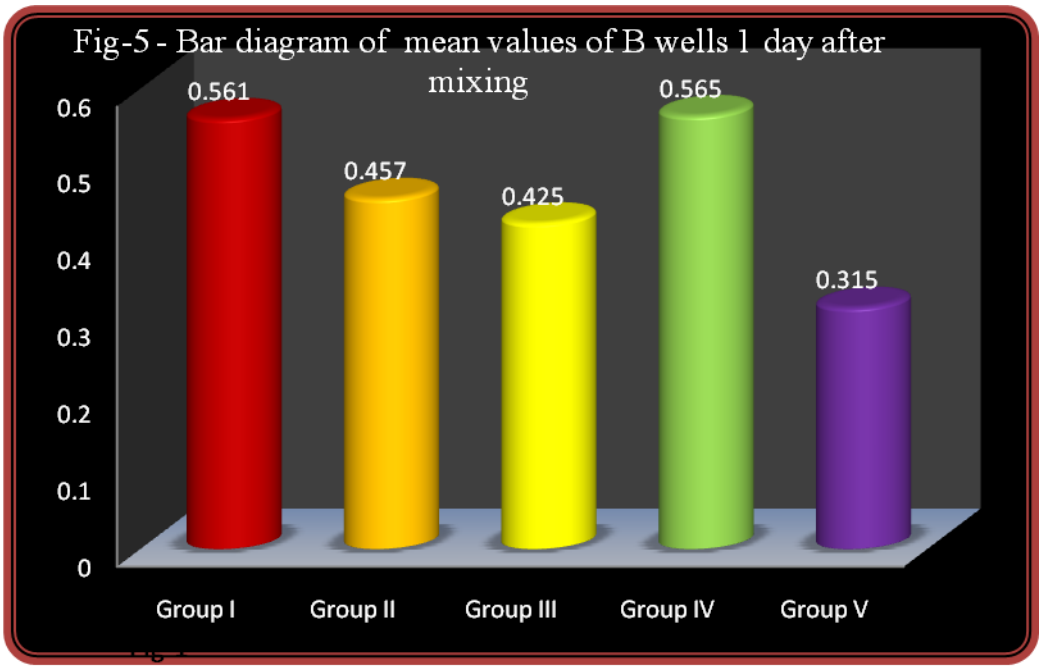


Fig-5 - Bar diagram of mean values of B wells 1 day after mixing
0.6
0.5
0.4
0.3
0.2
0.1
0
0.561
0.457
0.425
0.565
0.315
Group I
Group II
Group III
Group IV
Group V

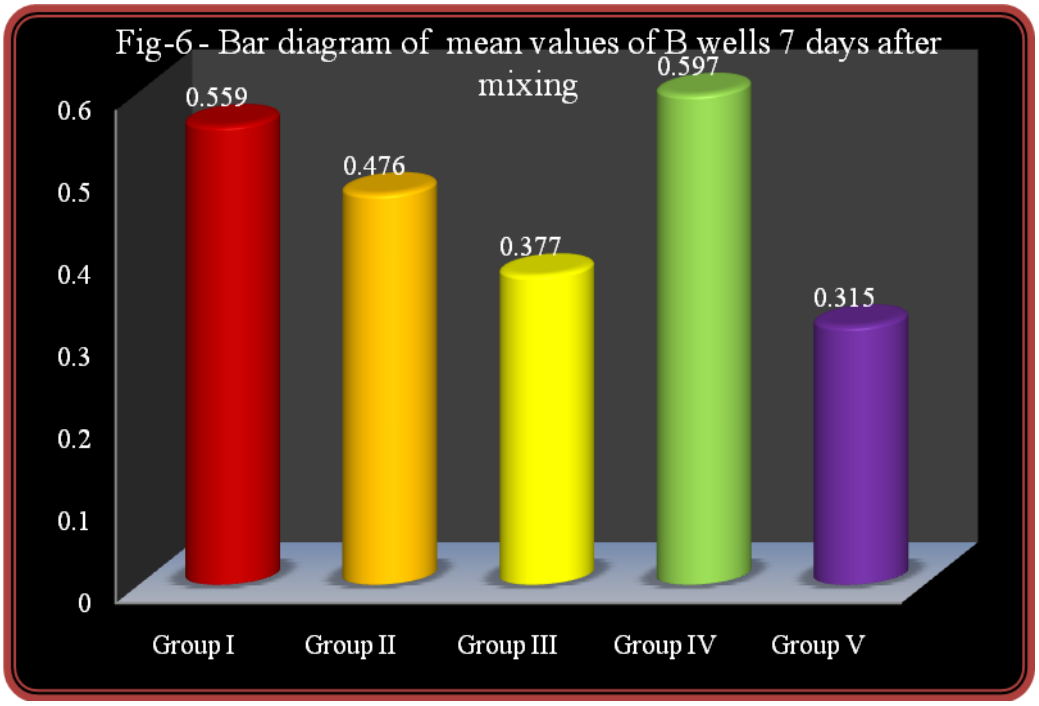


Fig-6 - Bar diagram of mean values of B wells 7 days after mixing
0.6
0.5
0.4
0.3
0.2
0.1
0
0.559
0.476
0.377
0.597
0.315
Group I
Group II
Group III
Group IV
Group V

The results of the Direct Contact Test of endodontic sealers for the time periods of 20mts, 1day and 7 days are shown in bar diagrams 1, 2, 3, 4, 5& 6.

In wells A, where bacterial growth was observed in the presence of the tested material;

Group IV (Tubli-Seal EWT) showed constant and complete inhibition of the bacterial growth throughout the incubation period of 7 hours at 20 minutes, 1 day and 7 days.

Group I (Sealapex) showed inhibition of the bacteria initially and decreased antibacterial activity at 1 day and 7 days respectively.

Group II (RoekoSeal) inhibited bacteria only in the 20 mts sample followed by a decrease in its antibacterial activity at 1day and 7days.

Group III (EndoRez) did not show any statistically significant antibacterial activity at 20 minutes, 1 day or 7 days.

The results of B wells, in which the transferred bacteria were incubated in the absence of the tested materials measuring the short-term

direct contact effect, did not differ for Group II (RoekoSeal) and Group III (EndoRez).

Group IV (Tubli-Seal EWT) showed constant and complete inhibition of the bacterial growth throughout the incubation period of 7 hours at 20 minutes, 1 day and 7 days.

Group I (Sealapex) showed inhibition of the bacteria initially and decreased antibacterial activity at 1 day and 7 days respectively.

Discussion

It has been known for more than a century that bacteria colonize the root canal space [4]. The role of these bacteria and their by-products in the initiation and progression of pulpal and periapical diseases has been well established. Biomechanical cleaning and shaping, followed by the three-dimensional obturation of the root canal space are the common procedures used to achieve this goal. However, studies by Lin et al [32]and Sequeira et al [43] have demonstrated that part of the root canal space often remains untouched during chemomechanical preparation regardless of the techniques and instruments employed.

Love et al[31], Molander et al[36] and Sundqvist et al [45] reported the presence of microorganisms in areas such as the isthmuses, ramifications, apical deltas, canal space irregularities and dentinal tubules even after thorough chemomechanical preparation of the root canal system. It has also been postulated by Bystrom and Sjogren et al[5] that if these microorganisms persist in the root canal at the time of root filling or if they penetrate into the canal after filling, there is a higher risk that the treatment will fail.

According to Grossman[16] the most important requirements of an ideal sealer are biocompatibility, excellent seal, adequate adhesion and antimicrobial property.

Rappaport et al [19] stressed on the fact that "The ideal root canal cement should be bactericidal". The need of the day is an endodontic sealer with strong antimicrobial properties but at the same time meet the requirements suggested by Grossman [6].The said sealer should also be biocompatible.

Leonardo and Leal (1991) had stated that to seal a root canal means to fill it in all its extension with an inert, antiseptic material, obtaining the most hermetic seal possible. The endodontic sealers enhance the possible attainment of an impervious seal by serving as filler for root canal irregularities and minor discrepancies between the root canal and the core material[36].

Zinc oxide eugenol is the most commonly used root canal sealer and has a successful clinical record. It has served as the benchmark with

which other sealants are compared, as it reasonably meets most of Grossman's requirements for sealers[22].

Luebke and Ingle in 1976 forecast a new paradigm for endodontics involving the use of calcium hydroxide in medicating and sealing the root canal[3]. This has led to the introduction of several calcium hydroxide based sealers.

Among the new root canal filling materials are the silicone-based (RoekoSeal) and the resin based sealers (Epiphany, EndoRez). RoekoSeal (RSA; Roeko, Langenau, Germany) is a Polydimethyl siloxane based root canal sealer with good adaptability, showing increased diffusibility and better sealing capacity in a dry environment. EndoRez is a dual cured methacrylate resin based sealer that is designed to bond to resin coated gutta percha for creating adhesion between the intraradicular dentin and the core root filling. The increased hydrophilicity is believed to enhance its penetrability into the dentinal tubules thereby also increasing its claimed antibacterial efficacy. Roeko Seal is a new material that includes particulate gutta percha in a poly dimethyl siloxane base [34].

The aim of this invitro study was to evaluate the antibacterial activity of four different endodontic sealers on Enterococcus faecalis by means of a direct contact assay. The sealers used were Sealapex (Calcium hydroxide based sealer), RoekoSeal (Polydimethyl siloxane based sealer), EndoRez (Urethane dimethacrylate resin), and Tubli-Seal EWT (Zinc oxide eugenol based sealer).

The Direct Contact Test (DCT) proposed by Weiss et al in 1996 has many advantages over agar diffusion test. It is a quantitative and reproducible assay which allows water insoluble materials to be tested. It relies on direct and close contact between the test microorganism and the material tested, being virtually independent of the diffusion properties of both the tested material and the media used. In addition to its reproducible and quantitative nature, the results of DCT unlike those of the Agar diffusion test (ADT), were not affected by the size of the inoculum, thereby facilitating the standardized measurements of a large number of specimens and their respective control simultaneously on the same microtitre plate. It also has the ability to monitor the bacterial growth, both in the presence and absence of the materials to be tested,

so in the present study the direct contact test was chosen as the appropriate method of testing the antimicrobial activity of sealers[6].

The results of this study were tabulated and analyzed by Kruskal Wallis One way Anova and Mann - Whitney 'U' Test. The highest antimicrobial property was shown by Zinc Oxide Eugenol sealer (Tubli-Seal EWT) (0.713, 0.808 and 0.759 at 20mts, 1day and 7days) followed by Calcium hydroxide based sealer (Sealapex) (0.676. 0.781 and 0.612 at 20mts, 1day and 7 days). No significant difference was seen between the Polydimethyl siloxane based sealer (RoekoSeal) (0.530, 0.561 and 0.487 at 20mts, 1 day and 7 days) and Urethane dimethacrylate based sealer (EndoRez) (0.386, 0.382 and 0.432 at 20 mts, 1 day and 7 days).

This variation in the antibacterial activity of each tested sealer with time interval is in accordance with previous studies by Weiss et al, Shalhav et al, Fuss et al, and Giuseppe Pizzoa[14, 15]. These may be attributed to the diffusion of antimicrobial components present in these sealers.

The present investigation showed Zinc oxide eugenol based sealer (Tubli-Seal EWT) to have the maximum antibacterial activity and statistically significant inhibition of bacterial growth throughout the study period of seven days, which is in accordance with the previous findings of Kont F, Kaplan AE, SequeiraJF, C. R. Sipert, and Giuseppe Pizzoa[29, 26, 43, 42,]

It has been established by Leonardo and Kont F[29] that eugenol is a potent antibacterial agent and is conceivable that it plays a major role in the antibacterial activity of Zinc Oxide Eugenol based sealers. Eugenol is bactericidal at relatively high concentrations being able to induce cell death and inhibit cell growth and respiration[29]. Hume has shown that in dentin immediately beneath the Zinc Oxide Eugenol the concentration of eugenol is sufficient enough to inhibit bacterial mechanism [40]. Furthermore, if the Zinc Oxide Eugenol contacts wet tissue, the eugenol concentration increases. This eugenol can inhibit white cell chemotaxis, synthesis of prostaglandins and nerve activity. Several biochemical mechanisms have been proposed by Markowitz et al to explain the

cytotoxicity of eugenol and its utilization in restorations to prevent bacterial penetration[40].

The calcium hydroxide based sealer; Sealapex showed antibacterial properties but to a lesser degree than the zinc oxide based sealer (Tubli-Seal EWT). This is in accordance to the studies of Fuss Z et al and Kayogulu G et al[6] who had concluded Sealapex to be mildly effective antimicrobial agents over short duration. Esterela et al[32] had hypothesized that in calcium hydroxide the antimicrobial mechanism is influenced by its speed of dissociation into calcium ions and hydroxyl ions. The antibacterial effect of Sealapex is also based on its dissociative ability into calcium and hydroxyl ions. This dissociation into hydroxyl ions creates a high pH (12.5) environment leading to decreased bacterial adherence to matrix extracellular proteins. It also inhibits the enzymatic activities that are essential for microbial metabolism, growth and cell division, thus rendering the environment unfavorable for the growth of microorganisms.

Brystom and Sundqvist [5, 37,] found that for calcium hydroxide sealers to be an efficient antimicrobial agent, it should maintain a pH

level greater than 12.5.As the calcium hydroxide sealers set the pH declines to 9.14, causing it to lose its effectiveness as Enterococcus faecalis can survive at a pH below 11.5[5,37].

Laboratory experiments to measure the radicular dentin pH have suggested an inadequate rise in the pH in dentinal tubules for effective results[37]. The limited antibacterial activity of calcium hydroxide sealer in the present study may be attributed to a lack of sufficient pH elevation, limited solubility and diffusibility of calcium hydroxide into dentinal tubules and possibly buffering ions present in the tubules[18].

RoekoSeal, which is a recently introduced Polydimethyl siloxane based sealer, showed a slight anti bacterial activity for the first 3 hours which drastically reduced over time[32]. According to Salome Egger et al the antibacterial activity may be attributed to the nano silver present in the sealer which is used as a preservative. The antibacterial activity may be related to the oligodynamic effect of heavy metal ions which exert a lethal effect on bacteria. The antibacterial activity of silver ions is due to its high affinity to cellular proteins. When these metal ions (silver) combine with sulfur groups, proteins are denatured. Denaturation occurs

because the bonding interactions responsible for the secondary structure (hydrogen bonds to amides) and tertiary structure are disrupted. Heavy metal salts act to denature proteins in the same manner as acids and bases. Since salts are ionic they disrupt salt bridges in proteins. The reaction of a heavy metal salt with a protein usually leads to an insoluble metal protein salt.

When a silver nanoparticle (AgNP3) is mixed with polydimethylsiloxane (PDMS) the resultant PDMS-AgNP3 combination shows good antibacterial property.

Studies by W R Moorer et al[47] have proved that even extremely small amounts of silver ions have significant harmful effects on bacteria. Hence it also provides a valuable alternative to the use of systemic antibiotics or disinfectants.

RoekoSeal is a new material that includes particulate Gutta-percha in a Polydimethyl siloxane base. W R Moorer et al[47] have found through microbiological analysis that a biologically active Zn^{2+} ion slowly leaches out from gutta-percha which produces an antibacterial activity.

In the present study RoekoSeal showed antimicrobial activity .The previous studies using ADT method of analysis showed no antibacterial activity both 24hrs and 7 days. But the DCT indicated that RoekoSeal had antibacterial activity in the freshly mixed samples. The results may be different due to the insolubility and no diffusion of the material in Agar medium[29].

EndoRez (Urethane dimethacrylate resin) based endodontic sealer has a hydrophilic nature which potentially improves its sealing property. Incubation of E. faecalis for1 hour at pH 3 and 3.5 showed that low pH alone does not have an impact on its viability. Slow setting, leaching of non reacted monomers and the lowest pH (below 4) are probably important for the continuing antibacterial effect of EndoRez[20].

However the present study which utilized EndoRez sealer did not show any significant antimicrobial activity. EndoRez was clearly sticky with a moist surface even 7 days after mixing, which indicates that the setting of the sealer was not yet complete at this point. The lower the wettability, the more the hydrophilic the substrates are, and the faster the liquid will spread on substrates and wet the surface. However,

hydrophilic surface characteristics of a sealer could facilitate the penetration of the sealer into the fine details of the root canal system but thereby positively affect their antibacterial effectiveness.

The sealers evaluated in this study showed different inhibitory effects which may be related to their different composition. Over all Zinc oxide eugenol based sealers and calcium hydroxide based sealers proved to be effective against the microorganisms at the varying time intervals studied. In the present study eventually all the sealers except Zinc oxide eugenol lost their antibacterial effect over the time period tested (20mts, 1 day and 7 days).

Thus the incorporation of antimicrobial components into root canal sealers may become an essential factor in preventing the regrowth of residual bacteria and control of bacterial re-entry into the root canal space and may also be of benefit in the treatment of persistent or recurrent infections.

However additional studies both in-vitro and in-vivo, are needed to evaluate the antimicrobial effects within dentinal tubules and biocompatibility of these sealers.

Summary

Antibiotic activity of endodontic sealers can improve the success rate of endodontic treatment provided the physical properties are not compromised. The dentin adhesive sealers are superior in case of manipulation, radio opacity, setting time, and excellent adaptation to canal walls, but the antibacterial activity of the Urethane dimethacrylate resin based and Polydimethyl siloxane based sealers is questionable. An in-vitro experimental study was formulated to evaluate the antibacterial activity of four endodontic sealers on Enterococcus faecalis by a Direct Contact Test.

The study materials grouped and selected were Group I, Calcium- hydroxide based sealer (Sealapex), Group II, Polydimethyl siloxane based (RoekoSeal) , Group III ,Urethane dimethacrylate resin based sealer (Endo Rez), Group IV Zinc Oxide Eugenol based sealer (Tubli-Seal EWT) . The sealers were mixed in strict compliance with the manufacturer's recommendations.

The direct contact test was performed based on turbidometric determination of bacterial growth in 96 well microtiter plates. The kinetics of the outgrowth in each well was monitored at 600 nm at

37^0C. Side walls of the microtiter plate wells were coated with freshly mixed tested material and a 10 µL bacterial suspension was placed. After 1 hr of incubation at 37°C which ensured direct contact between bacteria and tested material, Brain Heart Infusion broth (245 µL) was added to each of these wells and gently mixed for 2 min. These were designated as 'A' wells. 15 µL were then transferred from these A wells to an adjacent set of 4 wells containing fresh medium (215 µL) which were designated as 'B' wells.

The bacterial outgrowth was monitored both in the presence (A wells) and in the absence of the tested material (B wells). The recordings were based on the reading of the transmittance values in the spectrophotometer. Higher the transmittance value, the higher was the antimicrobial activity (i.e. less microbial growth).The microbial growth was recorded every 1 hour using a spectrophotometer for 7 hours at time intervals of 20mts, 1 day and 7 days.

The results obtained were subjected to statistical analysis by Kruskal Wallis One way Anova and Mann –Whitney 'U' test.

Conclusion

Under the limitations of this study, the following conclusions were inferred:

1. Endodontic root canal sealers had different inhibitory effects on Enterococcus faecalis during the growth period.

2. Calcium hydroxide based sealer (Sealapex) had an initial antibacterial activity for 10 hours, which slowly reduced with time.

3. Polydimethyl siloxane based (RoekoSeal) endodontic sealer underwent a brisk decrease in antibacterial activity after 3 hours followed by a decrease in its antibacterial activity at 1day and 7days.

4. Urethane dimethacrylate resin (EndoRez) based sealer had no antimicrobial property.

5. Zinc oxide Eugenol based sealer (Tubli-Seal EWT) was the most effective and Urethane dimethacrylate resin based sealer (EndoRez) was the least effective against Enterococcus faecalis.

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