Research Article

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Evaluation of the Anti Bacterial Action of Four Commercially Available Endodontic Sealants on *Entererococcus Faecalis* Using Direct Contact Test: An In Vitro Study

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ABSTRACT

Aim: To evaluate and compare in-vitro the antibacterial activity of four endodontic sealers (Sealapex (Calcium hydroxide sealer), Tubliseal (Zinc oxide eugenol sealer), RoekoSeal (Polydimethyl siloxane based sealers), and Endo Rez (Urethane dimethacrylate resin based sealer) Enterococcus faecalis by a direct contact test over a period of time.

Materials and Methodology: 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. 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.

Results: results are subjected to statistical analysis by Kruskal Wallis One way Anova and Mann –Whitney 'U' test.

Conclusion: Under the limitations of this study endodontic root canal sealers had different inhibitory effects on Enterococcus faecalis during the growth period and 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..

Keywords: Enterococcus faecalis, root canal, sealers, antibacterial, endodontic,direct contact test.

INTRODUCTION

Endodontics is a rapidly advancing clinical discipline of dentistry which is concerned with prevention and control of the root canal infection .W. D. Miller in 1894 first published his observations from root canals with infected pulp space. Since then bacteria has been implicated in infections of endodontic origin. Naidorf in 1972 stated that the necrotic pulp becomes a "privileged sanctuary" for clusters of bacteria and their by products. [1] The endodontic micro flora is typically a polymicrobial flora consisting 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. [2] Enterococcus faecalis is the most

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frequently isolated species from endodontic infections. The ability of E. faecalis to cause periapical disease and chronic failure of an endodontically treated tooth is due to its unique ability to bind to the collagen of the dentinal tubule and remain viable within the tubule. [1] E. faecalis posses certain virulence factors including lytic enzymes, cytolysin, aggregation substance, pheromones, and lipoteichoic acid. It has been shown to adhere to host cells, express proteins that allow it to compete with other bacterial cells, and alter host responses. [3] E. faecalis has the capacity to endure prolonged periods of starvation until an adequate nutritional supply becomes available. Once available, the starved cells are able to recover by utilizing serum as a source of nutrition. E. faecalis is able to form 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 organisms. [2]

Bystrom and Sunquist had observed that bacteria, which survive instrumentation and irrigation, rapidly increase in numbers in the empty canals in the period between appointments. Sunquist had recommended the use of anti-bacterial medicaments between appointments during root canal treatment of non-vital teeth. [5] An ideal root canal sealer in addition to its ideal "sealing" properties should leave the tooth in the most biologically inert condition possible and must prevent re infection and growth of any microorganisms remaining in the canal, thereby favoring periapical tissue repair. [4-5]

Many methods have been used to assess the antimicrobial efficacy of different root canal sealers and 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, measuring the effect of close contact between test bacteria and tested material based on the kinetics of bacterial growth became more popular. Moreover, it is a quantitative assay which allows insoluble materials to be tested. [6] In this invitro study the antimicrobial activity of four different endodontic sealers, Endo Rez (Urethane dimethacrylate resin based sealer) Tubliseal EWT (Zinc oxide eugenol sealer), Roekoseal (Polydimethyl siloxane based sealers), Sealapex (Calcium hydroxide sealer) on Enterococcus faecalis based on the direct contact test is assessed and compared at time intervals of 20 minutes, 1 day and 7 days.

MATERIALS AND METHOD

A comparative in vitro study to evaluate the anti bacterial activity of four endodontic sealers on Enterococcus faecalis by a modified direct contact test was undertaken. Enterococcus faecalis pure strains ATCC 29212 were employed for testing the antibacterial activity of endodontic materials.

Table 1: 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 sulfonamide 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 and Nano silver (preservative)
3	UDMA resin-based, root canal sealer	Endo Rez (Ultradent)	Urethane dimethacrylate resin (matrix); Zinc oxide; Barium Sulfate and Resin pigments
4	Zinc oxide Eugenol based radiopaque sealer	Tubliseal EWT (SybronEndo)	ACCELERATOR:4-Allyl-2-Methoxyphenol 97-53-0 NA NA 24,Dimeric acid resin and mineral oil BASE :Mineral oil, barium sulfate, zinc oxide, lecithin, cornstarch

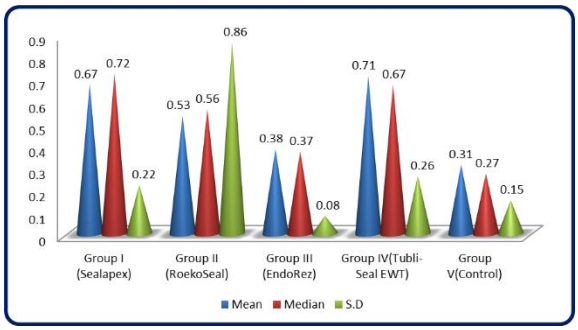

Fig 1: Group comparison using kruskal Wallis test 20 mts after mixing.

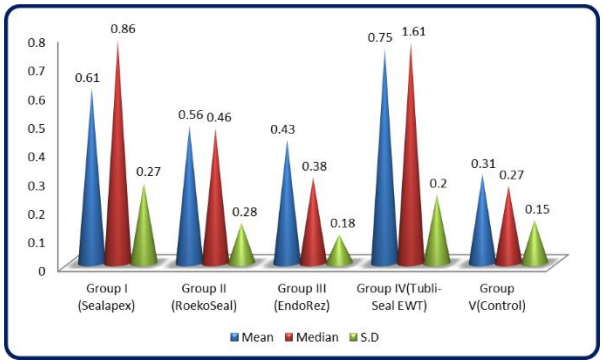

Fig 2: Group comparison using Kruskal Wallis test 1 day after mixing.

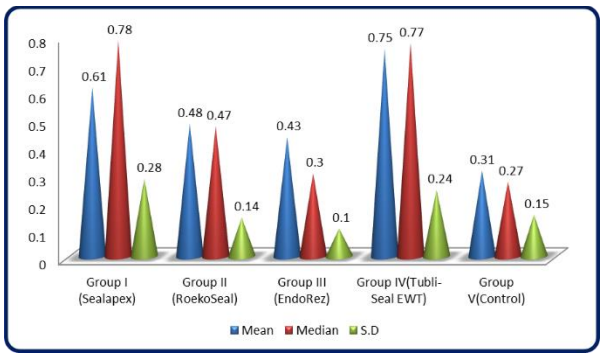

Fig 3: Group comparison using Kruskal Wallis test 7 days after mixing.

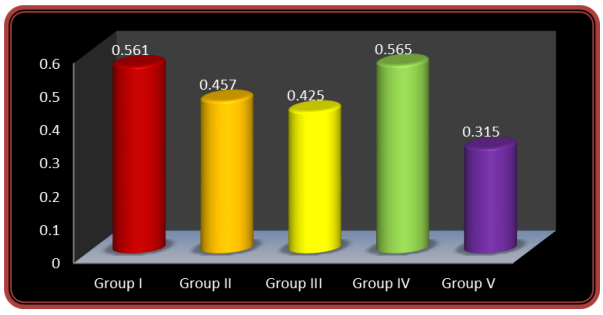

Fig 4: Mean value of A wells 20 mts after mixing.

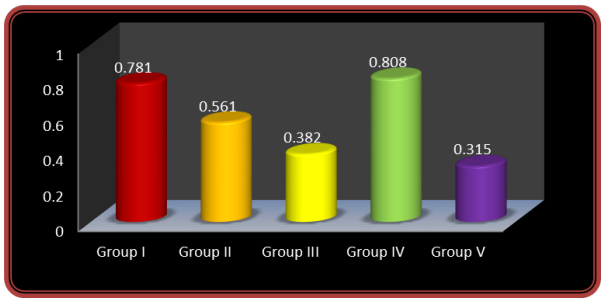

Fig 5: Mean value of A wells 1 day after mixing.

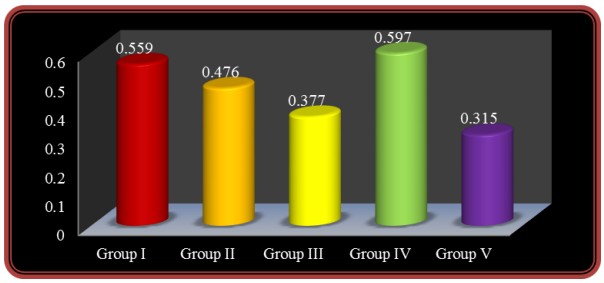

Fig 6: Mean value of A wells 7 days after mixing.

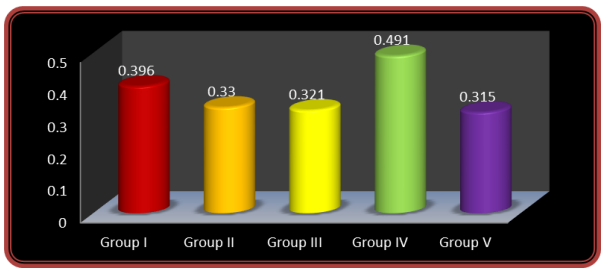

Fig 7: Mean value of B wells 20 mts after mixing.

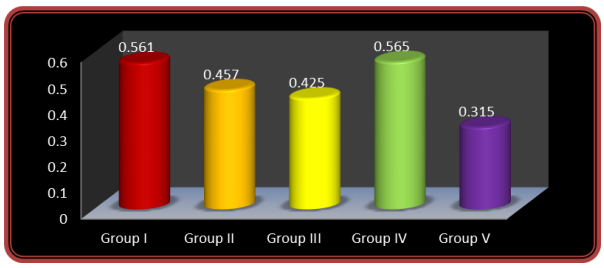

Fig 8: Mean value of B wells 1 day after mixing.

Preparation of medium for E.feacalis:

Brain Heart Infusion Broth M210-110G:

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.

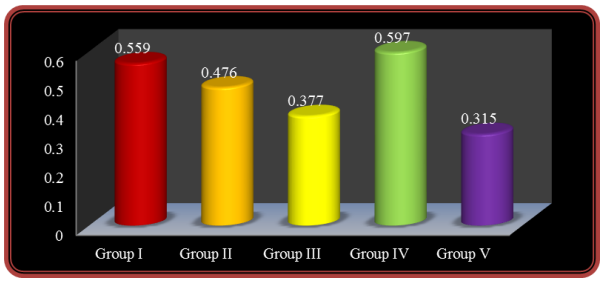

Fig 9: Mean value of B wells 7 days after mixing.

Table 1: 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

Table 2: Inter group comparison using Mann Whitney 'U' test1 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

Table 3: 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

Preparation of specimens:

Bacteria were grown aerobically from frozen stock cultures in brain heart infusion (BHI) broth at 37°C. Cells were harvested by centrifugation and re suspended in fresh medium. Inoculums were prepared by the re suspension 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. 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 37^0C and recorded every 1 hr using a spectrophotometer. 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).

After 20 min, a 10 µL bacterial suspension (10^6 bacteria) was placed on the test 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. 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

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 Bio-Rad spectrophotometer. Data was recorded and statistically analyzed using Kruskal Wallis One way Anova and Mann –Whitney 'U' test.

Fig.1 - 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).

Fig.2 - 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).

Fig.3 - 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.1- 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.2 - 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.3 - 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.

The results of the Direct Contact Test of endodontic sealers for the time periods of 20mts, 1hr and 7 hours are shown in Fig 4-9. The columns are representative of the mean values of the optical density of the groups over a seven hour period.

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 (Fig 4), 1 day (Fig 5) and 7 days (Fig 6) . 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, (Fig 7), 1 day (Fig 8) and 7 days (Fig 9). Group I (Sealapex) showed inhibition of the bacteria initially and decreased antibacterial activity at 1 day and 7 days respectively

DISCUSSION

Studies by Lin et al[7] and SequeiraJF et al[8] have demonstrated that part of the root canal space often remains untouched during chemo mechanical preparation regardless of the techniques and instruments employed. Love et al[9], Molander et al[10] and Sunquist et al[11] reported the presence of microorganisms in areas such as the isthmuses, ramifications, apical deltas, canal space irregularities and dentinal tubules even after thorough chemo mechanical preparation of the root canal system. It has also been postulated by Bystrom and Sjogren et al 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.[5] The present investigation showed Zinc oxide eugenol based sealer (Tubliseal EWT) to have the maximum anti bacterial activity and statistically significant inhibition of bacterial growth throughout the study period of seven hours, which is in accordance with the previous findings of Kont F[14], Kaplan AE[15], SequeiraJF[16], C. R. Sipert, and Giuseppe Pizzoa.[17]

It has been established by Leonardo and Kont F 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 a bactericidal at relatively high concentrations being able to induce cell death and inhibit cell growth and respiration.[15] Hume has shown that in the dentin immediately beneath the Zinc Oxide Eugenol the concentration of eugenol is sufficient enough to inhibit bacterial mechanism. 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.[4]

The calcium hydroxide based sealer; Sealapex showed antibacterial properties but to a lesser degree than the zinc oxide based sealer (Tubliseal EWT). This is in accordance to the studies of Fuss Z et al and Kayogulu G et al who had concluded Sealapex to be mildly effective antimicrobial agents over short duration.[18] Esterela et al 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 .[17-20]

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. 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 much 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.[20] 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 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 have found through microbiological analysis that a biologically active Zn^{2+} ion slowly leaches out from gutta-percha which produces an antibacterial activity [17].

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. [15]

Endo Rez (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 Endo Rez 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.

CONCLUSION

Under the limitations of this study, the following conclusions were drawn: Endodontic root canal sealers had different inhibitory effects on Enterococcus faecalis during the growth period. Calcium hydroxide based sealer (Sealapex) had an initial antibacterial activity for 10 hours, which slowly reduced with time. 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. Urethane dimethacrylate resin (EndoRez) based sealer had no antimicrobial property. 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.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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