Comparative study of three calcium hydroxide based root canal sealers using different cultivating techniques.

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Abstract: The aim of this study was to evaluate the antimicrobial properties of three calcium hydroxide based endodontic sealers: Apexit sealer (Ivocalr/Vivdent, Schaan and Liechtenstein), CRCS sealer (Coltene-Whaledent, U.S.A) and Sealapex sealer (Kerr Co, Italy) against *Streptococcus α haemolyticus* (facultative anaerobic bacteria). **Materials and Methods**: Three methods were used to evaluate the antibacterial activities of the three root canal sealers against *Streptococcus α haemolyticus* using "Agar diffusion test". The strains were prepared and inoculated into 5ml broth and incubated at 37^{o}C for 24 h. The freshly mixed sealers were placed into the prepared wells of agar plates (inoculated with the test microorganisms). The antimicrobial effect of each sealer was determined by measuring the diameter of zones of inhibition in millimeters at one and three days period. Five plates were prepared for every sealer in each method. **Results** showed that Sealapex gave the highest mean of inhibition zone diameter. This was followed by CRCS and Apexit showed the lowest mean of inhibition zone after one day. While at the three days period, the Sealapex gave the largest inhibition zone diameter and there was no statistically significant difference between CRCS and Apexit groups. The three methods used confirm these results.
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Key words: Root canal sealers, *Streptococcus α haemolyticus,* Agar diffusion test

1.Introduction

Among the ideal criteria set up by Grossman in the 1980's, for the selection of root canal sealer; *"It must possess bactericidal properties or at least has bacteriostatic effect"* Fraunhofer and Branstetter (1982).Flora of root canal infections are polymicrobial. While some are asymptomatic, others are associated with serious infections. The seriousness of an infection beyond the apex of a tooth is related to number, virulence, host resistance, and associated anatomic structures. If the infection spread beyond the tooth socket, it may localize or continue to spread through the bone and soft tissues as diffuse abscess or cellulitis, Khemaleelakul et al.,(2002).

Many researchers investigated the composition of the microbiata from acute and chronic endodontic infections. Fox and Isenberg(1967) studied the bacterial populations of 381 positive cultures from root canals and their antibiotic resistance pattern. They found that incidence of *Enterococci* which was second to *Viridans streptococci* in the study, Fox and Isenberg(1967). Ibrahim and Aisa(1973) found that 58.8% (204 out of 381) pure positive cultures taken from infected root canals before treatment were *Streptococci.*Ibrahim and Aisa (1973) . Infective endocarditis caused by the *Viridans* group of organisms is generally a result of their invading into the blood stream during intra oral procedures; tooth extraction, endodontic treatment, or even during tooth brushing,Samaranayake(1996).The *Viridans* group or other species of *Streptococci* typically shows alpha hemolysis on blood agar. Various biological properties of calcium hydroxide are due to its strong alkalinity, (pH 12.5). Thus, several bacterial species commonly found in infected root canals are eliminated after short period when in direct contact with this substance. Most of the endo-pathogens are unable to survive in such highly alkaline environment, Siqueira and Lopes (1999). Mirijana and Branka(2006) evaluated the antimicrobial activity of five root canal sealers: AH26 (a resin based paste);Apexit (calcium hydroxide based paste); Endo-methasone and Tubliseal (zinc oxide eugenol-based materials) and Ketac Endo Aplicap, (glass ionomer based sealer). Antimicrobial activity was tested against *S. mutans* 70C and *L.casei* ATCC 27773 using ADT (agar diffusion inhibitory test) on TYC SB, blood and MRS agars. The results confirmed that epoxy resin and zinc oxide-eugenol based sealers had the greatest antimicrobial effect. Calcium hydroxide and glass ionomer based sealers showed significantly lower antimicrobial activity compared to AH26, Endo-methasone and Tubliseal. The greatest antimicrobial activity was found for epoxy resin based sealer (AH26) for both tested microorganisms. In several studies, the antibacterial activity of calcium hydroxide[$Ca(OH)_2$]containing sealers was tested by 'Agar diffusion test' (Al-Khatib et al.(1990);. Therefore this study aimed to evaluate the antibacterial effect of three $Ca(OH)_2$ based sealers using different cultivating techniques.

2.Materials and methods

2.1.Materials

2.1.1. The root canal

The root canal sealers studied were:

1. Apexit sealer (Ivocalr/Vivdent, Schaan and Liechtenstein).
2. CRCS sealer (Coltene-Whaledent, U.S.A).
3. Sealapex (Kerr, Italy)

All of them were mixed according to the manufacturers' instructions.

2.1.2. Microorganism:

Microorganism used :in this study was *Streptococcus α haemolyticus*.

It is facultative anaerobic bacteria.

2.2.Methods:

Three methods were used to evaluate the antibacterial activities of the three root canal sealers against the *streptococcus α haemolyticus.*

The strains were prepared and inoculated in 5ml broth and incubated at 37^{o}C for 24 h.

2.2.1.Method (1)

1ml of the prepared bacterial suspension was impregnated and mixed by hand shacking with the blood agar while it is still liquid, then poured into sterile Petri dishes. Plates were left for 5 minutes to allow the media to harden. Wells were then punched using sterile disposable micropipette tips of 5mm diameter and 5mm depth in each agar plate and filled with the freshly mixed sealers.

2.2.2.Method (2)

Mueller-Hinton Agar was used instead of blood agar plates. Typical formula of Mueller-Hinton Agar by g/l contains: beef, dehydrated infusion from 300.0, casein hydrolysate 17.5, starch 1.5, agar 17.0, supplemented with yeast extract 0.1% and glucose extract 0.1%. Seeding was done using sterile cotton swabs, by spreading/brushing the bacteria across the surface of the blood agar, vertically and horizontally from the bacterial suspension. Similar to that tube # 3 of the Mc Farland scale. Plates were left for 5 min to allow the absorption of the inoculum.

2.2.3.Method (3)

Petri dishes containing blood agar were prepared .Seeding was done using sterile cotton swabs, by spreading/brushing the bacteria across the surface of the blood agar, vertically and horizontally from the bacterial suspension. Similar to that tube # 3 of the Mc Farland scale. Plates were left for 5 min to allow the absorption of the inoculums. Wells of 5mm diameter and 5mm depth were punched in each agar plate and filled with the freshly mixed sealers.

The agar plates were incubated into anaerobic jars at 37^{0}C for 24 and 72 hrs. The antimicrobial effect of each sealer was determined by measuring the diameter of zones of inhibition in millimeters. Five plates were prepared for every sealer in each method.

3.Results

3.1. Results of Blood agar impregnated with 1 ml of bacterial suspension (Method 1)

(Blood agar impregnated with 1 ml of bacterial suspension-method 1) at one day period showed that there was a statistically significant difference between the three groups (P < 0.001) by ANOVA test.

Duncan's test showed that Sealapex gave the statistically significantly highest mean inhibition zone diameter. This was followed by CRCS. Apexit showed the statistically significantly lowest mean as shown in figures (26, 27, and 28)

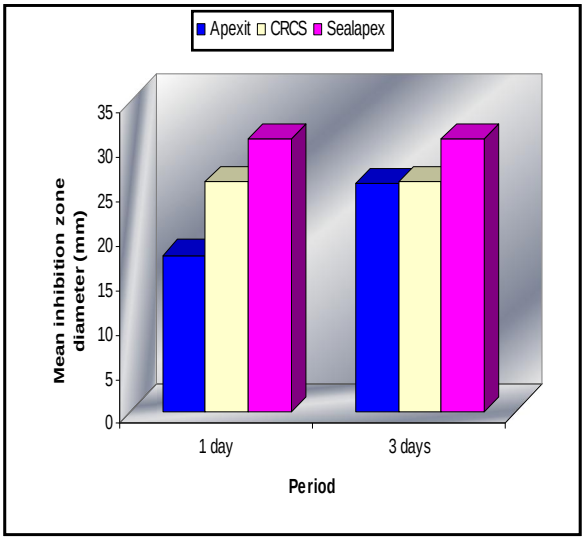
While After 3 days period ANOVA test showed that there was a statistically significant difference between the three groups (P = 0.037).

Duncan's test showed that Sealapex gave the statistically significantly highest mean inhibition zone diameter. There was no statistically significant difference between CRCS and Apexit which showed the statistical significant lowest means of inhibition zones.

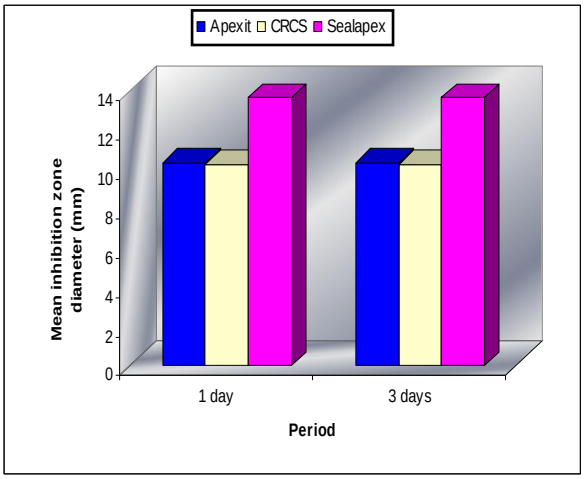
3.2. Results of Meuller Hinton agar (method 2)

After 1 day and 3 days of incubation ,ANOVA test showed that there was a statistically significant difference between the three groups (P < 0.001).

Duncan's test showed that Sealapex gave the statistically significantly highest mean inhibition zone diameter. There was no statistically significant difference between CRCS and Apexit which showed the statistical significant lowest means as shown in figures (1,2,3).



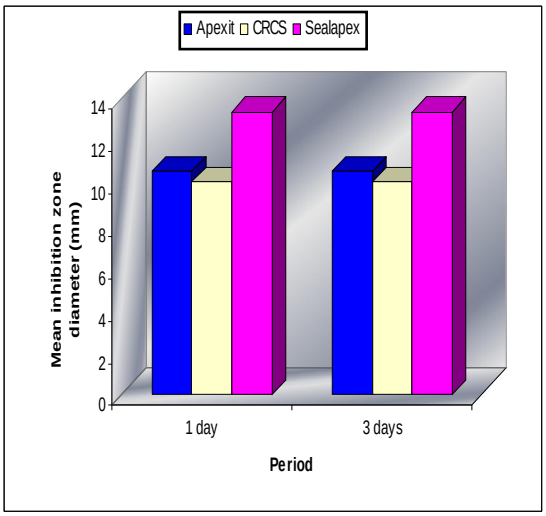
Mean inhibition zone diameter in the three groups (method 1) Fig (1)



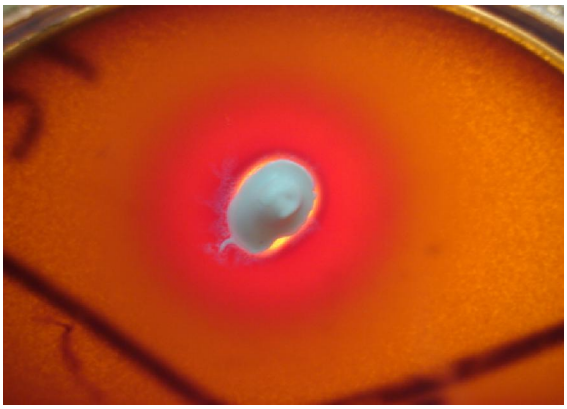
Mean inhibition zone diameter in the three groups (method 2) Fig (2)

3.3.Results of Blood agar with bacterial swab (method 3) :

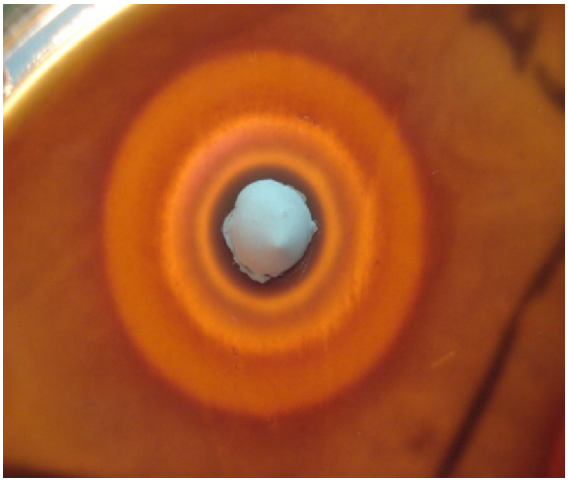
After 1 day and 3 days incubation, ANOVA test showed that there was a statistically significant difference between the three groups ($P = 0.009$). Duncan's test showed that Sealapex gave the statistically significantly highest mean inhibition zone diameter. There was no statistically significant difference between CRCS and Apexit which showed the statistical significant lowest means as shown in fig (3).



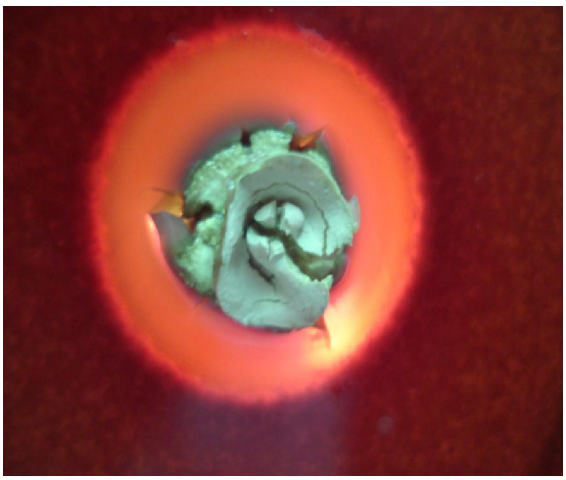
Mean inhibition zone diameter in the three groups (method3) Fig (3)



Inhibition zone produced by Apex



Inhibition zone produced by CRCS.



Inhibition zone produced by Sealapex

4. Discussion

The endodontic microbiota of untreated teeth was dominated by strict anaerobic bacteria, Siqueira (2000) .However, aerobic and facultative anaerobe microorganisms are usually minor constituents of primary infections, but they have been found in cases in which the treatment had been protracted, in flare-

ups, and associated with endodontic failures. These microorganisms can invade the root canal system during the treatment, survive the treatment procedures, and persist after obturation. Therefore, they cause secondary infections, Waltimo et al.,(1997); Sundqvist et al.,(1998); Siqueira(2000).

So, it is important to evaluate the antimicrobial activity of endodontic materials against aerobic and facultative anaerobic microorganisms.

The microorganism used in this study was *Streptococcus α haemolyticus*. It is facultative anaerobic bacteria. It includes two main groups, which were: *Viridans* group and *Pneumococci* group. The *Viridans* group of *Streptococci* principally lives in oropharynx and the oral cavity. It comprises roughly one-quarter of the total cultivable flora from supra-gingival , gingival plaque, and one-half of the isolates from the tongue and saliva. Thus, they can easily release to the blood stream during dental procedures, and sometimes during tooth brushing. Usually the bacteria released during dental procedures settle on damaged heart valves causing infective endocarditis(60% of the cases are due to this organism),Samaranayake (1996).

Three methods of the agar diffusion test (ADT) were used for antimicrobial evaluation of these tested sealers for the confirmation of the results.

The three methods revealed that after 24 h duration, Sealapex gave the highest mean inhibition zone followed by Apexit and CRCS. Where there was no statistical difference between their means. However, the means of inhibition zones by Apexit were slightly bigger than those of CRCS. This may be due to the high molecular size of CRCS and thus the low diffusion of the antimicrobial component of the sealer. CRCS contains zinc oxide, eugenol, eucalyptol, and small components of calcium hydroxide, Fuss et al.,(1997) .

Its antibacterial effect is probably due to the eugenol content rather than to the calcium hydroxide, Al-Khatib et al.,(1990).

Blood agar impregnated with 1ml of bacterial suspension gave the same results but with higher values in the mean inhibition zone diameters of the three groups in the 24 h period. This is probably due to more inoculum's number. As the microorganisms was totally mixed with the blood agar. Thus the bacteria were available in the whole thickness of the agar, increasing the sealer/ agar contact. Again in the 24h period Sealapex gave the highest statistically significant mean diameter of inhibition zone (30.6 mm), followed by CRCS (25.8mm) and then Apexit (917.6 mm) which showed the lowest mean inhibition zone diameter. At the three days period, the mean inhibition zone diameter of Sealapex and CRCS did not change. However, there was a significant increase in the inhibition zone diameter of Apexit group. This may be due to more diffusion of the material through the agar.

The anti microbial substance must diffuse through the aqueous agar medium and thus only the water soluble agents can be tested, Samaranayake(1996). On the other hand, the results of the media Mueller-Hinton agar (method B) and blood agar (method C) with bacterial swabbing were almost the same in both 24h and 3 days periods.

This confirmed the results that Sealapex had the highest antimicrobial effect against *Streptococcus α haemolyticus*, followed by Apexit and CRCS. These results came in agreement with Estrela et al.(2000) ; Lai et al.,(2001) ; Saleh et al.(2004) and Sipert et al.,(2005).

On the other hand, these results were in consistent with Fuss et al.,(1997) ; Siqueira et al., ,(1997);Mirjana and Branka (2006).

Fuss et al.,(1997) concluded that CRCS had stronger antibacterial effect than Sealapex with fresh samples. They referred their findings to the antibacterial effect of the eugenol content of CRCS rather than the calcium hydroxide content. While, Sealapex is based on polymeric resin and calcium formula. Thus, needs more time for polymerization and the release of hydroxyl ions. Fuss and his colleagues used direct contact test, and recorded their results every 30 min for 15h while in the present study, ADT was used, and the inhibition zones were measured after 1 and 3 days ,Fuss et al.,(1997)

Mirjana and Branka (2006) reported no antimicrobial effect of Apexit against *S. mutans* (*Streptococcus α haemolyticus*) on both types of agar. This is possibly because they measure the inhibition zone exactly after the application of freshly mixed specimens. Also, Apexit is based on resin and calcium hydroxide. Thus time was needed for polymerization of the materials, and accordingly there will be no hydroxyl ions which elevate the pH and having its antimicrobial effect, Mirjana and Branka (2006).

Siqueira et al.(1997) concluded that Sealapex presented low antibacterial activity when exposed to human saliva. This decreased activity might be due to the saliva buffering system, which was provided by proteins and by phosphate and bicarbonate buffering system. Therefore, even if Sealapex released hydroxyl ions which elevated the pH, that might reach to the toxic levels of the bacteria present in saliva. So when these hydroxyl ions were exposed to large volume of saliva, it is likely that the chemical effect of calcium hydroxide be rapidly neutralized by the buffer system, Siqueira et al.(1997)

Conclusions

This study was conducted to compare the antibacterial activity of three calcium hydroxide

based sealers namely: Apexit, CRCS, and Sealapex were three methods were used to evaluate the antibacterial activity of the three sealers. Sealapex had the highest antimicrobial effect. While there was no change in the means of the inhibition zones after three days for the other sealers.

All the calcium hydroxide tested sealers release calcium ions and are able to provide an alkaline medium and thus having antimicrobial property.

The prolonged setting time of Sealapex may render it beneficial for the long-term antimicrobial effect, and thus provide high pH even after complete observation.

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