The biocompatibility and antibacterial action of three endodontic sealers

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ABSTRACT

Background: Root canal sealers are regularly used in root canal fillings. Bacteria can multiply and grow inside the root canal system.

Materials and Methods: Twelve albino rabbits were used for the implantation of the sealer materials inside the S.C. tissue of the animals for 3, 14 and 28 days for determination of tissue reaction. A Muller-Hinton culture medium was used for culturing the bacterial swabs for 24 and 48 hours at 37°.

Results: The ZOE sealer showed the severest inflammatory reaction initially, and all the sealer materials revealed good tendency for tissue healing after 28 days. The Dorifil sealer exhibited the highest antibacterial action against all the microorganisms tested.

Conclusion: The endodontic sealers used in this study showed different types and severity of inflammatory reaction with good tendency for healing. All the sealer material tested had antibacterial actio against the bacteria but with different capacities.

Key words: Sealer, Bacteriology, Calcium Hydroxide, Epoxy. **(J Coll Dentistry 2005; 17(1):18-23)**

INTRODUCTION

The primary goal of successful endodontic therapy is complete obturation of the root canal space, after it has been adequately prepared and sterilized to prevent or eliminate any pathosis of endodontic origin. Solid core filling material such as gutta percha had been used to obturate the root canals in conjunction with a sealer to provide a hermetic seal and prevent apical leakage [1,2].

It is well established that the sealer cement is an extremely important component of the root canal filling in order to achieve a three-dimensional obturation of the root canal space.[3]

A wide variety of endodontic sealers are available to the profession, and these comprise sealers containing eugenol, and various medicated sealers like calcium hydroxide sealers and calcium phosphate cement, as well as epoxy resin containing root canal sealers [4].

All root canal sealers are required to possess certain physical and biological properties. These properties have been discussed at length in the literatures and include: Biocompatibility, strength, sealing ability, adequate working and setting times, low solubility, ease of manipulation, adhesion, antimicrobial effect, radiopacity, cytotoxicity and osteogenesis, and various other characteristics [5].

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Different products containing calcium hydroxide in their formulation have commercialized and recommended for use in endodontic therapy, due to their biocompatibility, animicrobial action, and ionic dissolution into calcium and hydroxyl ions. However, some of their properties, such as physical and mechanical properties of solubility and disintegration inside the canal, low strength, lack of adhesion to dentin walls, and microleakage have been the subjects of doubts [6,7].

The epoxy resin based root canal sealers have shown good biocompatibility, and well tolerated by the periapical tissues, with better sealing ability and lower rate of microleakage. However, the main disadvantage of the epoxy resin sealers is the lack of tissue repair stimulating action, that is present in the calcium hydroxide and calcium phosphate cement due to the absence of dissolution of calcium and hydroxyl ions from the sealer material, with minimal antimicrobial action[8,9].

The aim of this study was to evaluate the biocompatibility to the surrounding living tissue (Histopathological study), and the antibacterial action against some microorganisms (Bacteriological Study).

MATERIALS AND METHODS

Histo-pathological Study

This study was done to evaluate the Biocompatibility and tissue damaging effects

of the tested sealer materials (Apexit, Dorifill, and Experimental sealers); implanted in the subcutaneous tissue of rabbits with the aid of polyethylene tubes for 3, 14 and 28 days.

Twelve Albino active female rabbits of comparable weight were selected and used. The rabbits were anesthetized by intra muscular injection of a mixture of 3 ml Ketamine, and 0.5 ml Zylesin in the thigh region. The dorsal skin was shaved and disinfected thoroughly with 5% iodine in alcohol.

Under aseptic condition, an incision of approximately 15 mm length was cut in the skin of the dorsal surface using scalpel and surgical blades No.15. After flap reflection, three separate sub-coetaneous pockets were prepared by blunt dissection to a depth of 20mm with a 30mm distance between each implant. The PE tubes were filled with the freshly mixed materials using a messing gun of comparable size. Each tube was filled completely and any excess material was removed carefully using sterilized gauze. Three PE tubes were used for each animal. The filled tubes were transferred to the site with the aid of straight tweezer. Each implant was inserted to the end of the separate pocket tunnel carefully.

After implantation, the wounds were sutured with two stitches for each incision line, using 3-0 black silk suture. Two ml of Amoxycilin (350 mg) was injected I.M, and antibiotic cream was applied topically to prevent post operative infection. All specimens were fixed in 10% buffered formalin. This procedure was repeated at each time interval.

The slides were examined under light microscope to evaluate the intensity and degree of inflammatory reactions around each tube end, and the subsequent tissue healing at the sites of implantation.

Bacteriological study

The antimicrobial action of the experimental sealer was studied, and its effect was compared with those produced by ZOE and CH sealers.

A Muller-Hinton culture medium was used for culturing the swabs. Three previously identified bacterial species were used for testing the anti bacterial ability of the three root canal sealers used. The bacterial species were:

1. Staphylococcus aureus (Gram + ve, facultative anaerobes).
2. Pseudomonas aeroginosa (Gram – ve, aerobic bacilli).
3. Escherichia coli (Gram – ve, aerobes).

All the bacterial strains used in this study are pure isolates provided by department of microbiological examination / Central Laboratory Of General Health / Ministry of Health. Each petri dish containing the media, was inoculated with a loop that previously immersed in the solution by usual streaking method. The streaking was done in three directions to ensure even distribution of the bacteria. Sterile absorbent filter paper disk of 5 mm diameter were impregnated with the freshly mixed sealer material, and immediately placed on the surface of the culture media. The assessment criterion is done by measuring the diameter of the inhibitory zone with a millimeter ruler around each paper disk, and the results were given in millimeters.

RESULTS

Histopathological study

1) Zinc oxide eugenol sealer Group

At three days, there was a severe acute inflammatory reaction (predominate neutrophil and macrophage), of necrosis areas containing pus. The surrounding area showed foam cells aggregation, with few fibroblast and congested blood vessels, figure 1.

At fourteen days, the sealer material appeared as fine dark black particles. It was surrounded by a thick dense fibrous tissue capsule, with no evidence of any chronic inflammatory reaction nor congested blood vessels, figurers 2.

At 28 days, the fibrous tissue capsule appeared thicker and more prominent in direct contact with the end of the tube, figure 3.

2) Experimental sealer group

At three days, mixed inflammatory tissue response, demonstrated by the infiltration of (neutrophil, macrophage), with few fibroblast cells. There is no congested blood vessels nor necrosis. The sealer material appeared as fine deposits, that aggregated predominantly at the material – tissue interface. Figure 4.

At fourteen days, loose connective tissue formation with few fibroblasts and thin collagen fibers, with small congested capillaries and few chronic inflammatory cells, with macrophages containing dark stain sealer material particles, and collagen fiber hyalinization. Figure 5.

At 28 days, a thin consistent fibrous tissue capsule that surround the end of the tube. Figure 6.

3) Apexit sealer group

At three days, the sealer material appeared as black and pinkish deposits aggregated at the tissue-material interface, that surrounded by collagen fibres with few fibroblast, Figure 7.

At fourteen days, dense cellular response that appear in its active stage, aggregation of thick bundles of collagen fibres, aggregation of foam cells, granulation tissue formation (scattered small blood vessels, chronic inflammatory cells infiltration, mainly plasma cells and lymphocytes). Figure 8.

At 28 days period, all the granulation tissue was completely replaced by a thick fibrous connective tissue capsule, with no evidence of inflammation. Figure 9.

Microbiology study

1) Staphylococcus aureus

The means and standard deviations of the calculations on ***Staphylococcus aureus*** were listed in table1, and represented in figure 10. From these table and figure, it is clear that Dorifil (ZOE) sealer exhibited the highest mean of inhibition zone value followed by the experimental sealer and Apexit sealer after 24 hours of incubation at 37°C. There is a very slight increase (1mm) in the action of all endodontic sealers tested after 48 hours.

Table 1: Means of zones of inhibition on Staph.

Sealer	24 hours		48 hours	
	Mean	S.D	Mean	S.D
Experimental	8	1.2	9	1.3
Apexit	7	1.1	8	1.3
Dorifil	14	3.1	15	2.9

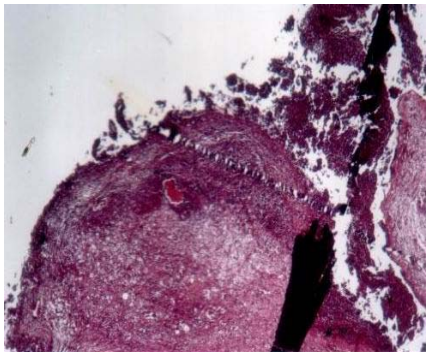

Figure 1 Dorifil after 3 days

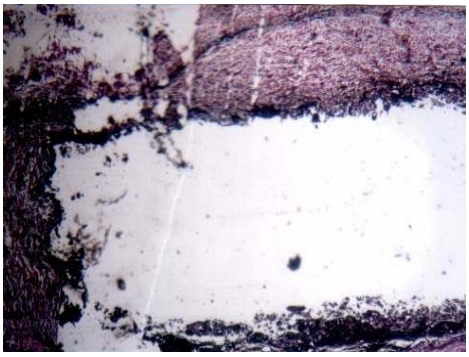

Figure 2 Dorifil after 14 days

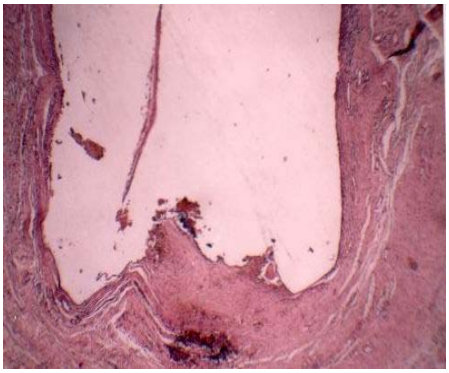

Figure 3 Dorifil after 28 days

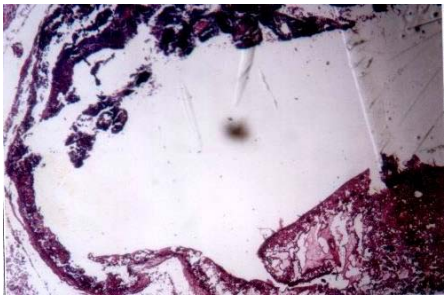

Figure 4 Experimental after 3 days

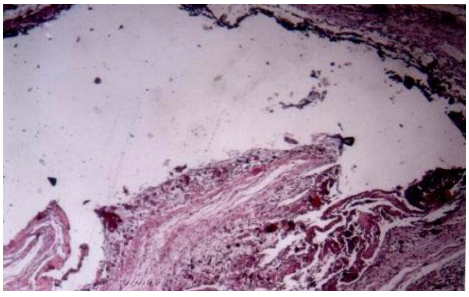

Figure 5 Experimental after 14 days

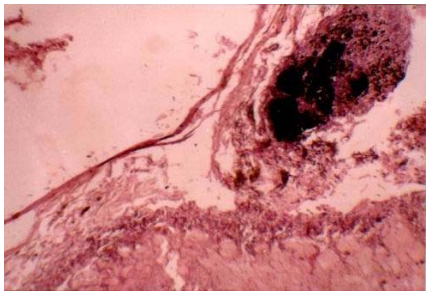

Figure 6 Experimental after 28 days

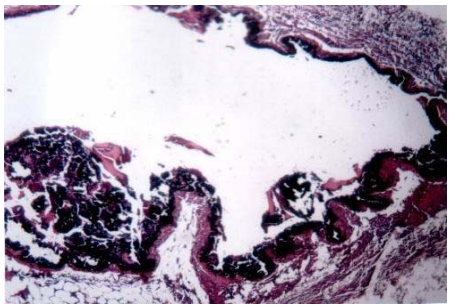

Figure 7 Apexit after 3 days

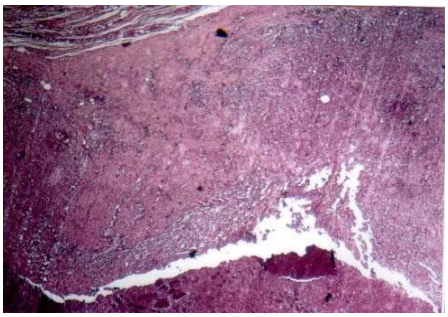

Figure 8 Apexit after 14 days

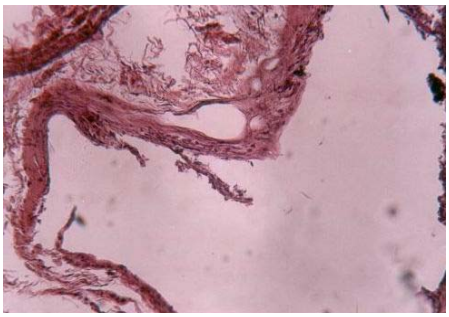

Figure 9 Apexit after 28 days

Figure 10 Inhibition zones on Staph.

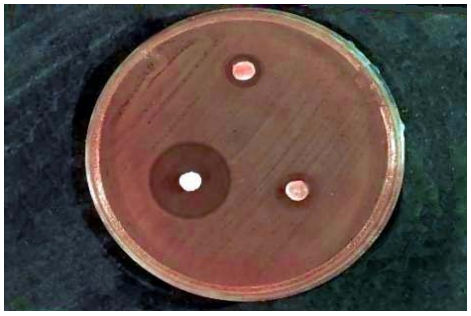

Figure 11 Inhibition zones on Pseudo.after 48 hours

2) E coli

The means and standard deviations of the three endodontic sealers susceptibility tests on *E.coli* were listed in table 2.

Table 2: Means of zones of inhibition on E.coli

Sealer	24 hours		48 hours	
	Mean	S.D	Mean	S.D
Experimental	9	2.2	9	2.3
Apexit	7	2.4	10	2.4
Dorifil	18	2.7	19	2.4

From table2, it can be seen that Dorifil (ZOE) sealer had the highest mean of inhibition zone against this microorganism after 24 hours and 48 hours. This is followed by Apexit (CH) sealer with a mean of which is the same after 24&48 hours. Experimental sealer came in the third step of this test.

Analysis of variance (ANOVA) test showed that there is a high statistical significant difference ($p<0.01$) between the three endodontic sealers in their antibacterial action against *E.coli.*

3) Pseudomonas aeruginosa

Table 3 represented the means and standard deviation of the antibacterial action of the tested root canal sealers on *Pseudomonas aeruginosa.* The results data showed that, Dorifil sealer had the highest antibacterial action against this Gram(-ve) aerobic bacteria.

Table 3 Means of zones of inhibition on Pseudomonus.

Sealer	24 hours		48 hours	
	Mean	S.D	Mean	S.D
Experimental	0	0	8	1.5
Apexit	0	0	10	2.1
Dorifil	22	4.4	24	3.2

On the other side, both Apexit and experimental sealer had no obvious antibacterial action on *Pseudomonas aeruginosa* at the first day, but their antibacterial effects were obvious after 48 hours of incubation, figure 11. Analysis of variance (ANOVA) test showed that there is a high statistical significant difference ($p<0.01$) between the three endodontic sealers.

DISCUSSION

Biocompatibility study

1) Three days period:

Among the sealers used in the present study, the most irritative material over the first observation period (3 days) appeared to be Dorifil that is a typical zinc oxide eugenol sealer.

Its irritative ability could be attributed primarily to eugenol and secondarily to the zinc ions that it contains. It should be noted that free eugenol is still present even after the sealer has been set and is available for release over a period, for this reason other investigators have suggested the substitution of eugenol by glycerin or even unsaturated fatty acids, such as linoleic and oliec [10,11]. The eugenol may act directly on the respiratory chain of cells, inducing irreversible cell change and necrosis.

Foam cell aggregation illustrates early body response to remove the lipid component of the sealer (Eugenol). On the other hand, the conventional calcium hydroxide sealer (Apexite) did not induce any inflammatory reaction that was observed in the samples taken from the implanted animals after three days of implantation with only thin collagen fibrils were observed. This is because of the fact that the release of calcium ions and hydroxyl ions was relatively low at the first three days. These

results make the Apexite calcium hydroxide sealer less irritant than the Dorifil zinc oxide eugenol sealer at three days period, this result is in agreement with [11,12] .

The experimental sealer that contain epoxy resin in addition to the calcium hydroxide, induced a mild mixed tissue response that is expressed through the infiltration of neutrophils, macrophages, and few lymphocyte with few fibroblasts at the three days period, this finding is in agreement with [13].

The addition of calcium hydroxide to the resinous sealer has contributed to the decreasing aggression of this resinous sealer [14], and has a satisfactory response in terms of tissue tolerance, a fact that is confirmed by this study.

2) Fourteen days period

After 14 days, Dorifil sealer appeared as dark black fine deposits, extend to the polyethylene tube. The Apexit calcium hydroxide sealer, induced a dense cellular with the formation of active fibroblasts, aggregation of thick bundles of collagen fibers, as well as the formation of granulation tissue. This finding is in agreement with [13]. Apexit sealer material produced a delay tissue response, with granulation tissue. The resinous experimental root canal sealer stimulated the formation of chronic inflammatory cells and loose connective tissue with few scattered congested capillaries in the site. The sealer material still appeared as dark stain deposits in the cytoplasm of the macrophages. These results are in agreement with [4,13, 15].

3) 28 days period

At the end of the experiment period (28 days), it seems that the healed tissue around the zinc oxide eugenol containing tubes is surrounded by thick fibrous capsules, free of inflammatory cells. This finding is in agreement with (Figueiredo et al 2001[13], Bernarth 2003 [4]).

On the other hand, calcium hydroxide revealed thick fibrous capsule without any type of inflammatory cells, this finding disagreed with (Figueiredo et al)[15] who reported that calcium hydroxide containing tubes were surrounded by a mild chronic inflammatory cells and giant cells beside the irregular calcifications. While (Zmener et al)[8] found an active fibrous tissue at 30 days which then become fibrotic as capsule after 90 days when calcium hydroxide sealer material was used.

After 28 days the entire inflammatory picture around the resinous experimental sealer was resolved and an well-organized thin fibrous capsule which was free of inflammatory cells was seen around the implants. This finding is in agreement with (Silva et al)[13].

Microbiology study

In this study, all sealer materials showed an inhibitory capacity with respect to the bacterial growth. Regarding staphylococcus aureus bacteria, all the sealers used inhibited the growth of bacteria but with varying degree in the first 24 hours: the greater inhibition obtained with Dorifil sealer, the lowest with Apexit. These results are in agreement with (Canalda et al) [16]. This is because Dorifil sealer contains Eugenol in its chemical structure. Eugenol is a chemical essence of oil of clove. Also, this high antibacterial potency of Dorifil may be due the inclusion of bismuth oxide (radiopacifier) in its chemical structure [17].

Apexit and the experimental sealers showed lower antibacterial action against staphylococcus aurous, in this study. Calcium hydroxide is strong alkaline substance that has a high antibacterial activity against oral bacteria.

The availability of hydroxyl ions is responsible for the high pH value. The low antibacterial action showed by Apexit and the experimental sealer may be due to its low diffusibility in agar artificial media which reduced its high pH and lowering its antibacterial activity [18].

In the experimental sealer, the addition of epoxy resin to the calcium hydroxide did not affect the antibacterial action of the later. The experimental sealer has approximately the same antibacterial activity of the Apexit sealer against al the microorganism tested in this study. This may explain that the addition of epoxy resin to the sealer contents did not impair the release of hydroxyl ions (as presented in the dissolution study) which are the responsible of the antibacterial action of the sealer.

For the E.coli bacteria, the same picture showed in Staph. Aurous was seen but with higher rate of growth inhibition for all types of sealer studied, with no significant difference in the zones of inhibition at the first and second days. This is due to higher diffusibility of eugenol than hydroxyl ions in the agar media and unaffection of eugenol by the buffering ability of the media like hydroxyl ions, these findings are in complete agreement with the findings of (Al-Katib et al)[17].

The antibacterial action of the root sealers used in this study against Pseudomonas aeruginosa bacteria is differed from that on Staph aureus and E. coli. Only in the second day of incubation, both the Apexit and the experimental sealers inhibited the growth of bacteria. This result is disagreed with (Al-Katib et al)[17] who did not find any difference in the antibacterial action of the calcium hydroxide sealer used in their study at 24 & 48 hours periods on Pseudomonas A.

This difference in the antibacterial action may be due the initial resistance of Pseudomonas A bacteria to calcium hydroxide material, unlike its high susceptibility to Dorifil zinc oxide eugenol sealer. The results of such antibacterial tests may not highly correlate with in-vivo data and what happens in the root canals or at the apex. Orstavic & Hapsalo [19] observed that serum and saliva can significantly diminish the effect of antibacterial agents.

It must be emphasized that endodontic filling materials with strong antibacterial activity, have frequently been found to induce an adverse effects during and after treatment and were also cytotoxic or even mutagenic [20]. The most desirable endodontic sealer would be one that combines maximal antibacterial effect with minimal toxicity.

REFERENCES

1- Hata G, Kawazone S, Toda T, Weine Fs. Sealing ability of thermoplasticized gutta percha fill technique as assessed by a new method of determination apical leakage. J Endod 1995; 21: 167-72.

2- Schafer E, Olthoff G. Effect of three different sealers on the sealing ability of both thermafil obturators and cold laterally compacted gutta percha. J Endod 2002; 28: 638-42.

3- Wu MK, Tigos E, Wesselink PR. An 18-month longitudinal study on a new silicon based sealer, RSA Roekoseal: A leakage study in vitro. Oral Surg Oral Med Endod 2002; 94: 499-502.

4- Huang Th, Kao CT , Chou MY. The biocompatibility of epxy resin based rot canal sealers in vitro. Int Endod J 2002;36: 345-9.

5- Caicedo R, Von Fraunhofer JA. The properties of endodomtic sealer cements. J Endod 1988; 14: 527-32.

6- Flax M, Barnett F, Transtad L. In vivo solubility of calcium hydroxide containing root canal sealers. J Dent Res 1986; 65: 298.

7- Siqueira FJ, Fraga Rc, Garcia PF. Evaluation of sealing ability, pH, and flow rate of three calcium hydroxide based root canal sealers. Endod Dent Traumatol 1995; 11: 225-8.

8- Zmener O, Maria BG, Cbrini RL. Biocompatibility of two calcium hydroxide-based endodontic sealers: A quantitative study in the subcutaneous connective tissue of the rat. J Endod 1988; 14:229-35.

9- Fonzi M, Fonzi I, Bertelli E. Biocompatibility and physico-mechanical properties of new venezia root canal sealer. Minerva Stomatol 2001;50: 91-100.

10- Gulati N, Chandara S, Jaiswal JN. Cytotoxicity of eugenol in sealer containing zinc oxide. Endod Dent Traum 1991; 7: 181-5.

11- Bernarth M, Szabo J. Tissue reactions intiated by different sealers. Int Endod J 2003; 36: 256-9.

12- Economoides N, Kotsaki VP, Rozos G, Shore R . Experimental study of the biocompatibility of four root canal sealers and their influence on the zinc and calcium content. 1995

13- Silva LA, Leonardo MR, Faciolli LH . Inflammatory response to calcium hydroxide based root canal sealers. J Endod 1997; 23: 86-90.

14- Barbara SV, Araki K. Cytotoxicity of some of canal sealers and their leachable components . Ora l Surg Oral Med Endod 1993; 75: 357-61.

15- Figueeiredo JA, Pesce Hf, Gioso Ma, Figueeiredo MA The histological effect of four endodontic sealers implanted in the oral mucosa: Submucous injection versus implant in poly ethylene tubes. Int Endod J 2001;34: 377-85.

16- Canalda C, Pumarola J. Bacterial growth inhibition produced by root canal seler cements with a calcium hydroxide base. Oral Surg Oral Med Endod 1989;68: 99-102.

17- Al-Alkatib ZZ, Baum RH, Morse DR. The antimicrobial effect of various endodontic sealers. Oral Surg Oral Med Endod 1990; 70: 784- 790.

18- Sjogren U, Figder D, Spangberg L. The antimicrobial effect of calcium hydroxide as a short term intracanal dressing. Int Endod J 1991; 24: 119-25.

19- Orstavic D, Hapsalo M. Desinfection by endodontic irrigants and dressing of experimentally infected dentinal tubules. J Endod Dent Traumatol 1990; 6: 142-45.

20- Nisengard RJ, Newman MG. Oral microbiology and immunology. 2nd Edition. WB Saunders Co. P 435; 1994.